

Phylogenetic and climatic constraints drive flowering phenological patterns in a subtropical nature reserve

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Abstract

Aims

Exploring flowering patterns and detecting processes are essential when probing into the nature of reproductive traits during the life history and the interactions among different evolutionary clades. Such patterns are believed to be influenced by many factors, but quantifying these impacts at the community-level remains poorly understood.

Methods

We investigated the flowering patterns based on long-term herbarium records in a given area from subtropical forest regions in southern China. We obtained 5258 herbarium voucher specimens collected from the Dinghushan National Nature Reserve (DNNR) belonging to 166 families, 943 genera and 2059 species and examined the month when each species was flowering during the period 1920–2007.

Important Findings

The results showed that plants flowered sequentially almost throughout the whole year, showing the characteristics of subtropical

evergreen broad-leaved forests. Flowering spectrums of the entire flora and the four life forms exhibited a clear unimodality that is probably typical of subtropical forest communities. Flowering patterns of the DNNR were positively correlated with mean rainfall, mean air temperature and mean sunshine duration. Median flowering dates of the 38 large species-rich families ranged from early April to late August; 25 families exhibited significant unimodal distribution patterns, whereas the remaining families were unclear or bimodal. Median flowering dates of the 10 most species-rich genera ranged from middle May to later July. While the results are consistent with climatic factors playing a general role in flowering patterns, median flowering dates varied significantly among species-rich families and genera, suggesting that phylogenies could provide specific constraints in subtropical forests.

Keywords: environmental driver, flowering phenology, phylogenetic effect, reproductive trait, subtropical forest

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INTRODUCTION

Exploring patterns of plant reproductive traits and detecting possible factors affecting these traits have been a focus of ecologists and botanists for over a century (Clarke 1893; Raunkiaer

1934; Steltzer and Post 2009; Swenson 2013; Westoby and Wright 2006). Flowering phenology is a key aspect since both temporal and spatial distribution of reproduction may be influenced by both phylogenetic and environmental factors (Li *et al.* 2015a, Li *et al.* 2015b). Phylogenetic conservatism

facilitates researchers making comparative investigations for specific plant traits on a temporal scale (Ackerly and Cornwell 2007; Chazdon *et al.* 2003). Flowering phenology is estimated to be conservative in light of the existence of phylogenetic constraints that influence patterns of forest succession and evolutionary processes of angiosperms (Kraft *et al.* 2008; Uriarte *et al.* 2010). Studies on natural sciences, including flowering phenology, are encountering a tendency to decline (Tewksbury *et al.*, 2014). Reproductive traits may be phylogenetically conserved, perhaps over millions of years and difficult to measure or quantify, but the interpretation of empirical analyses could still be usefully attempted (Pérez-Harguindeguy *et al.* 2013; Satake *et al.* 2013).

Both intraspecific and interspecific flowering events can be impacted by climatic shifts in temperature, rainfall, solar irradiance (Giuliani *et al.* 2014; Hamann 2004; Körner and Basler 2010; Ovaskainen *et al.* 2013). Flowering patterns of plant species could not independently exist apart from the effects of intrinsic genetic characteristics, which could also be explained with the help of biotic interactions from phylogenies (Elzinga *et al.* 2007; Frankie *et al.* 1974; Galloway and Burgess 2012). A well accepted distribution pattern is proposed as the unimodality (i.e. one significant peak during the whole flowering season), which is characteristic of plant communities located in lower latitudes (e.g. subtropics and tropics) (Corlett 1993). The well-known phylogenetic constraints hypothesis assumes that plants have a 'settled pattern' of flowering patterns; it begins with a particular time, develops for a period of time and fades to the end after full-blossoming stage (Robertson 1995). Though overlaps of flowering times may appear because of the presence of asynchronous rhythms of diverse species in a plant community, findings from many previous investigations agree that phylogenetic constraints play a role in the process of flowering events; community patterns (among diverse plant species) of flowering time are determined in large part by taxonomic membership (Clarke 1893; Kochmer and Handel 1986; Petanidou *et al.* 1995; Robertson 1924). Phylogenetic constraints can exist in the form of different evolutionary clades (at the hierarchy of family and genus) having closely related relationship. Examples of flowering phenology from temperate and tropical floras showed that flowering times of plant species are influenced by phylogenetic membership. Seasonal limitations of flowering times are caused by phylogenetic constraints, which may not have changed for millions of years (Kochmer and Handel 1986). Myrtaceae species sympatric to the temperate rainforest in Southern Chile were observed to have flowering periods patterns that constrained mainly by phylogenetic inertia at the family level (Smith-Ramirez *et al.* 1998). In Korea, a flora with 2867 angiosperm species showed that 63.8% of these species flowered in summer, followed by spring, autumn and winter. Flowering periods in summer and autumn were significantly longer than that in spring. Large families with more species differed in their flowering season and duration; both parameters were also segregated among genera within

these families. Both family membership and flowering season exhibited significant effects on flowering duration (Kang and Jang 2004), which demonstrates that the evolutionary influence on flowering time and duration can be observed at the community level when considering the interaction of flowering parameters and the taxonomic composition of species within plant communities. A study of the flowering and fruiting phenology for 20 dominant species of the xerophytic communities in the mountain-crest areas of the island of Majorca, Spain, showed that most flowering species were concentrated in the spring and early summer, ranging from March to November with a peak in June (Tébar *et al.* 2004). A repeated measures analysis that included 83 species from 32 genera demonstrated that congeners shared similar monthly distributions of flowering record on Barro Colorado Island (BCI), which indicated that phylogenetic effects on BCI flowering phenologies were strong within genera, but substantially weaker with families (Wright and Calderon 1995).

Recently, long-term records of plant species obtained from herbaria, museum and botanical gardens, combined with historical meteorological data, have been used to examine the impact of climate change on flowering trends. Such case studies are more common in both Europe (Fitter and Fitter 2002; Proença *et al.* 2012) and North America (Calinger *et al.* 2013; Lavoie and Lachance 2006; Miller-Rushing and Primack 2008), but the situation is different elsewhere, with phenological records usually highly dispersed, of short duration and limited to a few taxa. Attempts to utilize herbarium records are of significance when assessing community-level patterns of phenological responsiveness with broad species sampling and are crucial steps towards knowing about current and future impacts of climate change events on plant species responses. However, information from community-level flowering patterns of long-term herbarium records in a given area is still scarce; especially from subtropical forest regions such as China, which can strengthen our understanding processes of reproductive flowering events. Subtropical forests are characteristic of the transition from tropical flora to temperate zone, mainly preserved in China, ranging from 22°N to 34°N, with an area of about 2.5 million km² (more than one-fourth of land area in China) (Wu and Chen 2004). The Chinese subtropical forest region, the centre of diversity of the eastern Asian flora, harbours ~140 000 seed plant species from more than 2600 genera, many of which are endemic and of much conservation interest (Wu 1991). The existence of patterns or variation in flowering phenology among different evolutionary clades is poorly understood. The present study will add to our knowledge of flowering phenology in a Chinese subtropical forest, using flowering records from herbarium specimens collected in a homogeneous site spanning 88 years and including an interesting diversity of lineages.

In this study, we (i) explore the flowering characteristics of plant species in an insufficiently understood subtropical monsoon evergreen broad-leaved forest, (ii) test the hypothesis that an unimodal pattern is typical of plant communities in the

seasonal northern hemisphere and (iii) test the phylogenetic constraints hypothesis on the observed flowering patterns of closely related taxa in a subtropical forest. Furthermore, we proposed a hypothesis that climatic factors govern flowering patterns more generally (e.g. for the whole flora), while phylogenies constrain more specially (e.g. for several specific taxa).

MATERIALS AND METHODS

Study site

The Dinghushan National Nature Reserve (DNNR in short hereafter) (112°32'39" E to 112°35'41" E, 23°09'21" N to 23°11'30" N), established in 1956, was the first nature reserve in China and known as the 'green gem on the Tropic of Cancer'. The DNNR is located in Dinghu District, about 84 km from Guangzhou city in Guangdong Province of southern China, with an area of 1156 ha vegetation cover. It was also accepted as one of the first designated scientific research stations of the UNESCO 'Man and Biosphere' in 1979 (<http://www.unesco.org/mabdb/br/brdir/directory/biores.asp?code=CPR+02&mode=all>). The elevation ranges from 10 to 1000 m above sea level. The DNNR has a typical subtropical monsoon climate, with an annual average precipitation of 1678 mm, of which nearly 80% falls in the wet season (from April to September) and 20% in the dry season (from October to March). The mean annual temperature is 22.3°C and relative humidity is 77.7% (Zhou et al. 2011).

Treatment of flowering time

Original flowering time of the DNNR was recorded in three ways: definite flowering dates (e.g. April 21, 1921), with only flowering months indicated (e.g. April, 1921), or with indefinite flowering seasons (e.g. spring season, 1921). Given that flowering dates were inconsistently recorded, we transformed the flowering times lacking specific dates (i.e. months and/or seasons) to make these data comparable. For each season without a definite month, 3 months corresponding to the season (i.e. four flowering seasons were classified according the climatic conditions in the research area as follows: March to May as spring season, June to August as summer season, September to November as autumn season and December to the coming February as winter season) were used as an alternative; for each flowering month without a definite date, the median of a month (i.e. 15th) was regarded as an alternative flowering date, hoping to decrease possible bias resulting from different population sizes and sampling efforts and limit uncertainty regarding impacts of flowering duration by sampling at a specific area (Calinger et al. 2013; Miller-Rushing and Primack 2008). The midpoint is a conservative and reasonably robust estimate of median flowering time (Kochmer and Handel 1986). We explored frequency distributions of the median flowering time of the component species. Median flowering time for each species was defined as the midpoint of extreme recorded dates of flowering, with half of flowering records included before and after the midpoint.

Datasets

Specimens without precise data were discarded, and duplicates in the same collecting year and with the same species name were removed. A total of 5258 herbarium voucher specimens with flowering records were obtained, which were collected by dozens of botanists from 1920 to 2007 (collection data of voucher specimens from 2008 to 2013 were inaccessible in this study at this time). There were 2059 species (including subspecies, forms and varieties) that were mostly obtained from specimen records kept in the Herbarium of the South China Botanical Garden (IBSC), supplemented from a handbook of plant species and the e-flora database of the DNNR (http://www.efloras.org/browse.aspx?flora_id=620). These species belong to 166 angiosperm families according to APG taxonomic system (APG III 2009) and represented 95.4% of the total 2159 species documented in the DNNR. This work was conducted based on Forestry Standards for 'Observation Methodology for Long-term Forest Ecosystem Research' of the People's Republic of China (LY/T 1952-2011).

Analyses and statistical tests

To illustrate general flowering patterns of the DNNR, flowering records from the entire flora and the four life forms were used. Five items of information were taken from each specimen: family name, genus name, species name, life form and flowering time (i.e. specific date, month or season). Four types of life form were classified, i.e. trees (with 435 species), shrubs (with 389 species), herbs (with 942 species) and vines (with 293 species). For each type of life form, we quantified the number of species flowering in each month (from January to December). The curve fit of flowering spectrums for the entire flora and the four life forms were estimated using the quadratic model on SPSS (13.0) program. Furthermore, flowering dates of the entire flora and the four life forms were compared each other, and the statistical difference was determined using the analysis of variance (ANOVA) and least square difference (LSD) *post hoc* tests.

To correlate climatic factors with flowering patterns, we used data collected for rainfall, sunshine duration and air temperature that were obtained at the field atmospheric station of the DNNR. The climatic data used in our present study only covered the years for 1975–1995, this concentration helping to make our subsequent analyses relatively reasonable, since ~30% of specimens were collected during this period. The number of flowering species (the entire flora and four life forms) was correlated with these three climatic factors at the monthly scale using the Pearson test on SPSS (13.0) program.

To test the phylogenetic constraints hypothesis in the DNNR, flowering records from both confamilial and congeneric species were used. A number of 38 families containing ≥ 15 species per family (Online Supplementary Material), and the 10 most species-rich genera from seven families (with ≥ 10 species and ≥ 30 flowering records per genus) (Table 1) were selected to quantify potential phylogenetic constraints at the family and genus levels. We used this subset containing 1489 species (with 3982 specimen records) to avoid biasing statistical tests with samples of widely varying size, inexact estimation of flowering times and

Table 1: multiple comparisons of median flowering dates of the entire flora and the four life forms (showing *P* values) in the Dinghushan National Nature Reserve, southern China

	All species	Trees	Shrubs	Vines
Trees	0.001**			
Shrubs	0.093	0.080		
Vines	0.803	0.038*	0.448	
Herbs	0.013*	0.0001***	0.001**	0.157

The mean difference is significant at the 0.001 (***), 0.01 (**) and 0.05 (*) levels.

the many other difficulties that might result from having many categories with small samples sizes. The subset included 72.32% of all flowering angiosperm species in the DNNR. For each species-rich family and genus, we list flowering dates of herbarium records, and transformed each flowering date into the Julian calendar date in the year (365 days/year). Multiple comparisons between 703 family and 45 genus pairs were obtained, and the statistical difference among species-rich families and genera was determined with using the ANOVA and LSD *post hoc* tests on SPSS (13.0) program. All Figures were produced by 'lattice' library in R-package.

RESULTS

Flowering characteristics of the entire flora and the four life forms

The flowering spectrum at different hierarchical levels (i.e. the entire flora, the four life forms, the 38 large species-rich families and the 10 large species-rich genera), in most cases, exhibited relatively similar patterns in the DNNR. Among all the records of flowering date, the earliest one (the first 10 days of January) was for a tree species (*Prunus salicina*) from Rosaceae, and the latest one (the last 10 days of December) for herbaceous species (*Hedyotis auricularia* var. *mina*) from Rubiaceae, using the Julian calendar date in the year (365 days/year). The flowering period in the flora could last for greater than 11 months. Median flowering date of the entire flora (2059 species) was around July 27 in the DNNR. Averagely, approximate 438 plant species flowered each month during the period of 1920–2007.

Flowering spectrums of the entire flora and the four life forms in the DNNR exhibited significant unimodal distribution patterns (Fig. 1). The observed unimodal flowering patterns fit well when using the quadratic model, with high coefficients of determination for the entire flora (0.956), trees (0.798), shrubs (0.800), herbs (0.824) and vines (0.875) (Table 1). A peak of the flowering process was observed in July for the entire flora (with 683 species flowering), whereas in May for shrubs (with 165 species flowering), in June for trees and vines (with 106 and 92 species flowering, respectively), and in July for herbs (with 362 species flowering) (Figs 1 and 2). One-way ANOVA test results showed

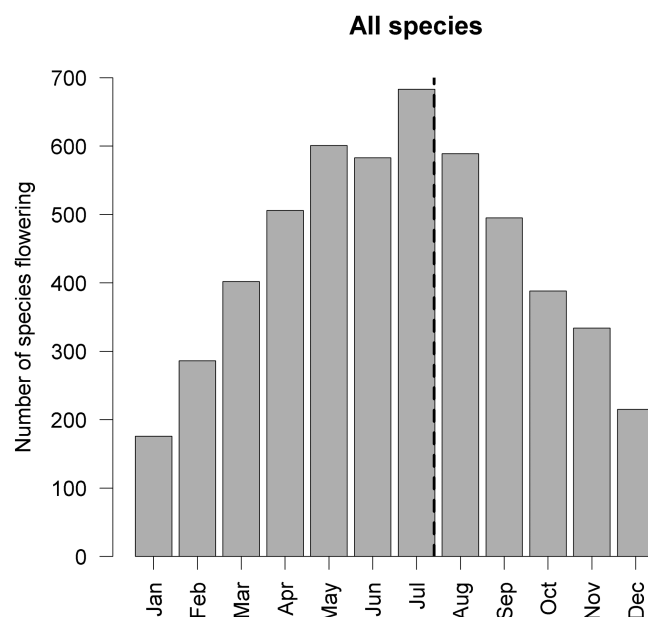


Figure 1: flowering spectrum of the entire flora in the Dinghushan National Nature Reserve. The dotted line represents the midpoint of flowering time.

significant difference among the entire flora and the four life forms ($df = 4$; $F = 6.407$; $P < 0.0001$). Median flowering date of trees was around July 3, followed by vines (July 9), shrubs (July 21) and herbs (August 3); and five item-pairs showed significant difference ($P < 0.05$).

Flowering characteristics of species-rich families and phylogenetic constraints

Most of (25) families exhibited a significant unimodal distribution pattern, whereas the remaining families were unclear (e.g. Acanthaceae) or bimodal (e.g. Asteraceae) (Fig. 3). Median flowering dates of the 38 large species-rich families were variable, ranging from April 8 to August 28 (Online Supplementary Material), specifically 1 family in April, 6 families in May, 14 families in June, 14 families in July and 3 families in August. One-way ANOVA test results showed significant difference in flowering time among species-rich families ($df = 37$; $F = 8.234$; $P < 0.001$). Approximately 47% of the 703 family pairs showed significant difference ($P < 0.05$).

Flowering characteristics of species-rich genera and phylogenetic constraints

The 10 most species-rich genera were sequentially listed according to median flowering dates. In the 10 genera, significant difference of flowering times was detected, with median flowering date ranging from middle May (*Ilex*) to late July (*Clerodendrum*), which coincided with the expected general patterns of flowering events concentrated in the spring and autumn seasons, but exhibited significant nonoverlapping of median flowering dates for some species-rich genera. Genera *Ilex* and *Carex* tended to flower in late spring; *Lasianthus*,

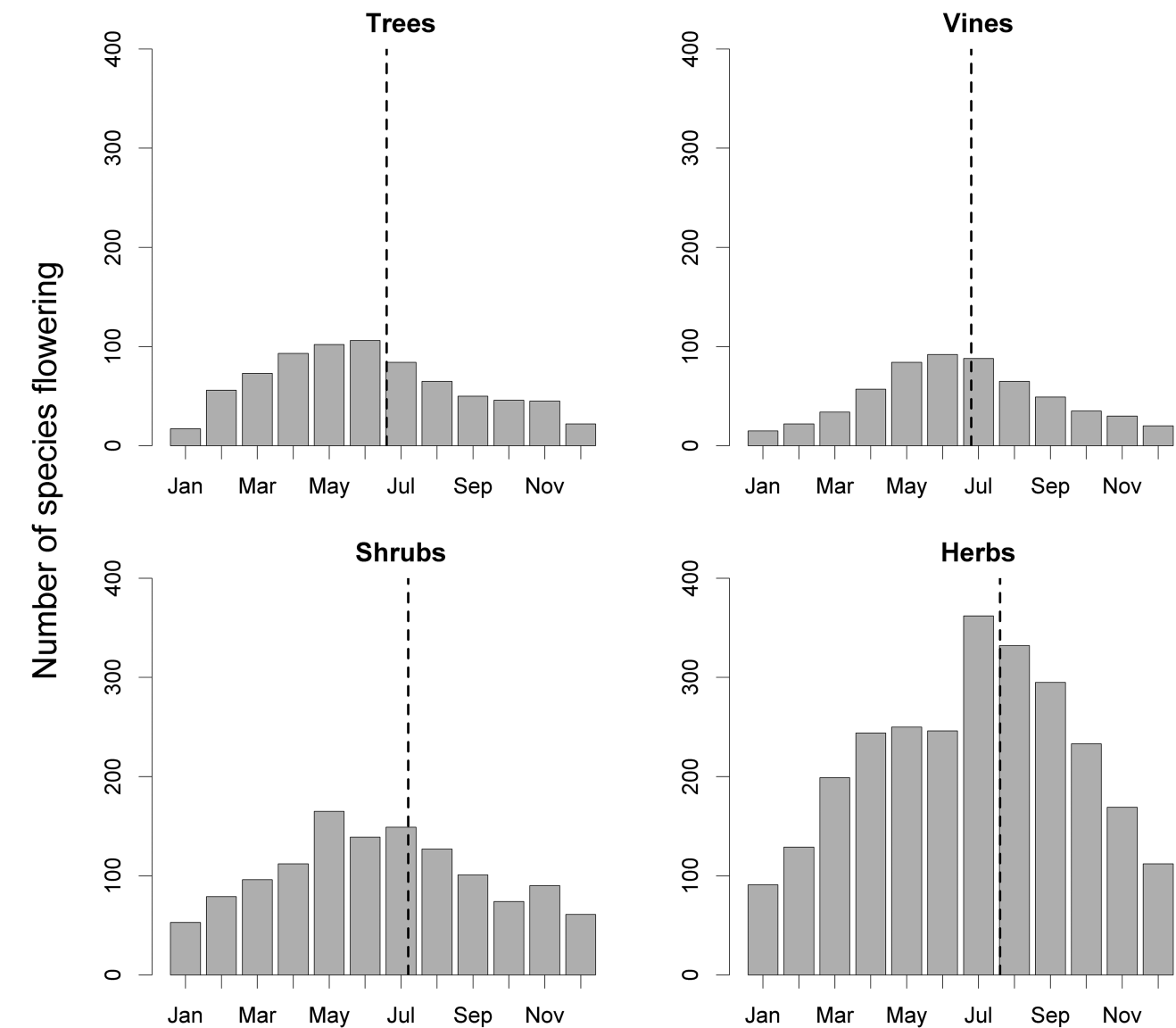


Figure 2: flowering spectrum of the four life forms in the Dinghushan National Nature Reserve (DNNR). The dotted line represents the mid-point of flowering time.

Solanum, *Cyperus* and *Polygonum* in early summer; while *Hedyotis*, *Ficus*, *Fimbristylis* and *Clerodendrum* in middle summer (Fig. 4; Table 2). One-way ANOVA test results showed significant difference in flowering time among species-rich genera ($df = 9$; $F = 3.332$; $P = 0.001$). Approximately one fourth of the 45 genus-pairs showed significant difference ($P < 0.05$).

Correlations of flowering patterns with climatic factors

This study showed that flowering patterns of the entire flora and the four life forms in the DNNR were positively correlated with mean rainfall (Pearson r ranging from 0.83 to 0.96) and mean air temperature (Pearson r ranging from 0.64 to 0.94), with all P values < 0.05 (Table 3). The flowering patterns of the entire flora and three life forms (i.e. herbs, shrubs and vines) showed weakly or moderately positive correlation

with mean sunshine duration, but displaying no statistical significance (Table 3). Exceptionally, flowering patterns of trees showed slightly negative correlation with mean air temperature, but displaying no statistical significance (Pearson $r = -0.17$, $P = 0.587$).

DISCUSSION

Flowering patterns and climatic factors in subtropical forests

Plant species flowered sequentially almost throughout the whole year in this study, which coincided with the basic characteristics of evergreen broad-leaved forest in a lower subtropical region, southern China. Generally, the study found a unimodal distribution pattern on flowering spectrums of the entire flora and four life forms when using a quadratic model.

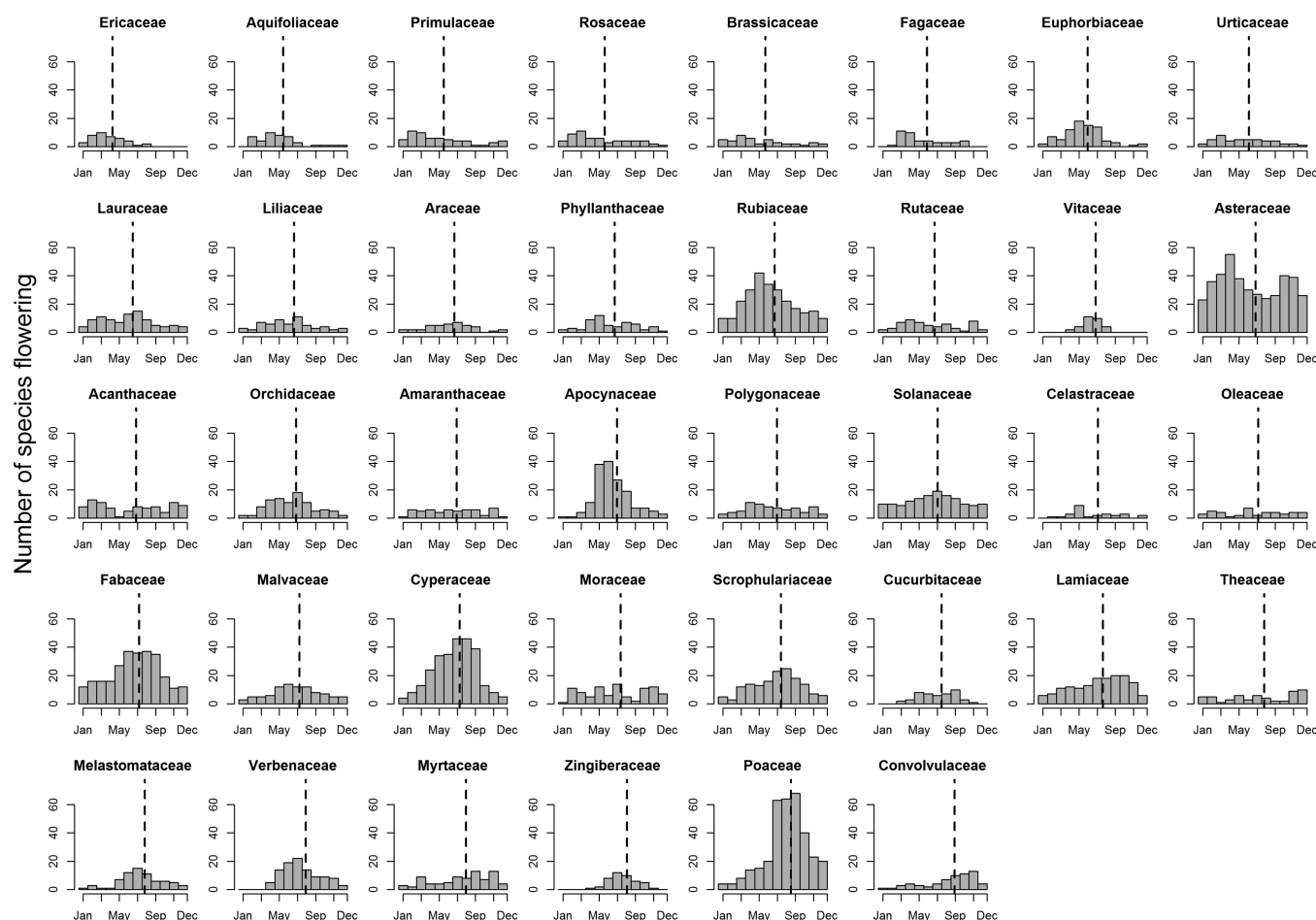


Figure 3: flowering spectrum of 38 species-rich families in the Dinghushan National Nature Reserve. The dotted line represents the midpoint of flowering time. Insert figures are presented according to the flowering time.

Further, we inferred that a unimodal distribution pattern was probably characteristic of the DNNR in south China. Our results basically supported a previous finding proposing that unimodality is typical of plant communities located in the seasonal northern hemisphere (Corlett 1993), though flowering patterns might shift along with the changes of climatic factors. Furthermore, our results showed that among the 38 most species-rich families, over two thirds of items exhibited a unimodal distribution pattern, whereas the remaining exhibited an unclear distribution pattern. Interestingly, several families (e.g. Asteraceae) flowered with a relatively clear bimodality (April and October), which might be partially explained by the more perennial species in autumn season rather than in spring. The findings were reasonable since some biological taxa might incongruently shift their phenological patterns by virtue of changes of in the physical environment of a natural community (Ovaskainen *et al.* 2013; Rudolf and Singh 2013). The above nonuniform results from species-rich families in the DNNR that still maintain a majority with unimodality imply that a diverse subtropical forest could still preserve its biological characteristics such as flowering phenology, presumably because of a stable community composition (Walters

et al. 2013). However, we need more case studies to deduce whether unimodality is a general phenomenon in subtropical forests.

Rainfall and air temperature were previously reported to be primary meteorological factors affecting flowering phenology (Sherry *et al.* 2007; Steltzer and Post 2009; Wolkovich *et al.* 2012). This study supported this point, provided direct evidence showing that both mean rainfall and mean air temperature might shape unimodal flowering patterns as detected in the forest region. Interestingly, we found that trees and vines (canopy species) tend to flower earlier, whereas shrubs and herbs (understory species) tend to flower later in the forest, which seemed to be reasonable since canopy species could obtain more and longer solar irradiance.

Historical influences could also be the reason. The DNNR, located in a lower subtropical monsoon climatic region, has a well-preserved vegetation landscape, good hydrothermal conditions, no wildfires or anthropogenic disturbance in the past four hundred years (Ye *et al.* 2008), which allow the presence of stable forest processes, including flowering phenological events. Our results implied that rainfall, air temperature

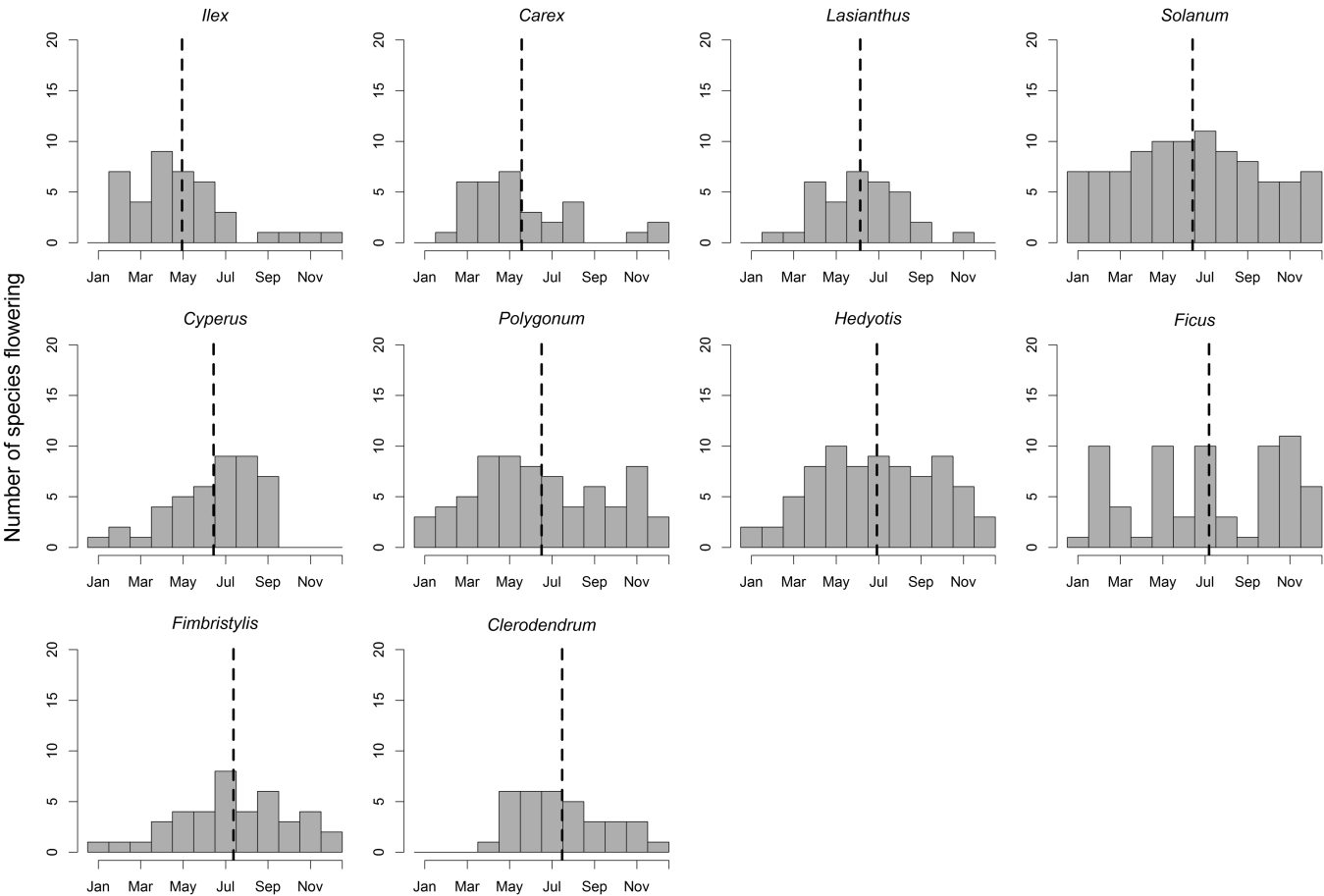


Figure 4: flowering spectrum of 10 species-rich genera in the Dinghushan National Nature Reserve. The dotted line represents the midpoint of flowering time.

Table 2: flowering date sets and *post hoc* tests of 10 species-rich genera in the Dinghushan National Nature Reserve, Southern China

Genus	Family	No. of species	No. of flowering records	Medium flowering date	Julian calendar date (mean ± SD)	<i>Ilex</i>	<i>Carex</i>	<i>Lasianthus</i>	<i>Solanum</i>	<i>Cyperus</i>	<i>Polygonum</i>	<i>Hedyotis</i>	<i>Ficus</i>	<i>Fimbristylis</i>
<i>Ilex</i>	Aquifoliaceae	27	40	May 14	133 ± 74									
<i>Carex</i>	Cyperaceae	17	32	June 2	153 ± 80	0.330								
<i>Lasianthus</i>	Rubiaceae	16	33	June 19	170 ± 59	0.070	0.433							
<i>Solanum</i>	Solanaceae	13	97	June 27	178 ± 98	0.006	0.153	0.632						
<i>Cyperus</i>	Cyperaceae	14	44	June 28	179 ± 63	0.014	0.190	0.632	0.940					
<i>Polygonum</i>	Polygonaceae	29	70	June 30	181 ± 94	0.005	0.126	0.532	0.821	0.910				
<i>Hedyotis</i>	Rubiaceae	17	77	July 13	194 ± 86	0.000	0.023	0.172	0.218	0.356	0.355			
<i>Ficus</i>	Moraceae	23	70	July 21	202 ± 105	0.000	0.008	0.081	0.083	0.179	0.162	0.611		
<i>Fimbristylis</i>	Cyperaceae	17	41	July 27	208 ± 82	0.000	0.007	0.060	0.066	0.129	0.118	0.422	0.717	
<i>Clerodendrum</i>	Lamiaceae	11	34	July 30	211 ± 65	0.000	0.007	0.052	0.058	0.111	0.101	0.356	0.612	0.881

Values in bold in the seventh and eighth columns represent statistically significant differences in median flowering dates between two genera detected at the 0.05 level using the LSD test.

and sunshine duration played a significant role in flowering patterns of the entire flora and the four life forms in the DNNR, which supports our hypothesis that climatic factors might govern flowering phenology generally.

Flowering phenology and phylogenetic constraints

Testing the phylogenetic constraints hypothesis without knowledge of the underlying evolutionary mechanisms may

Table 3: correlations of three meteorological factors and the number of species flowering each month in the Dinghushan National Nature Reserve, Southern China

Factor	Mean rainfall	Mean air temperature	Mean sunshine duration
Trees	$r = 0.83$ $P = 0.0008^{***}$	$r = 0.64$ $P = 0.026^*$	$r = -0.17$ $P = 0.587$
Shrubs	$r = 0.90$ $P = 0.00007^{***}$	$r = 0.80$ $P = 0.0019^{**}$	$r = 0.21$ $P = 0.510$
Herbs	$r = 0.87$ $P = 0.0002^{***}$	$r = 0.94$ $P = 0.0000^{***}$	$r = 0.50$ $P = 0.098$
Vines	$r = 0.95$ $P = 0.0000^{***}$	$r = 0.86$ $P = 0.00029^{***}$	$r = 0.30$ $P = 0.34$
The entire flora	$r = 0.96$ $P = 0.0000^{***}$	$r = 0.92$ $P = 0.00002^{***}$	$r = 0.32$ $P = 0.301$

The correlation is significant at the 0.001 (***), 0.01 (**) and 0.05 (*) levels (two-tailed).

still be informative, if we can understand how plant traits correlate to proximate phenological responses at the community level (Diez *et al.* 2012). In a temperate Japanese secondary forest, flowering processes for species with a shorter flowering duration was more synchronous (Osada *et al.* 2003). In a Mediterranean shrub community, regional differences (either ecological or biogeographical) were found to be important in determining flowering time along with possible phylogenetic constraints (Petanidou *et al.* 1995). This study confirmed the presence of phylogenetic constraints and detected the effects on flowering phenologies at the scales of family and genus. In the DNNR, flowering times of the 38 species-rich families varied; most of families lasted for several months, whereas some flowered year-round (e.g. Rubiaceae, Orchidaceae, Liliaceae, Lauraceae and Cyperaceae, etc.). Nearly half of median flowering dates of these families showed significant difference, from early April to late August. These results from long-term herbarium records provide direct and strong evidence from a subtropical flora for the phylogenetic constraints hypothesis.

In the Carolina and Japanese temperate floras, the 10 largest genera from the Asteraceae differed significantly in median flowering time, which can be viewed as evidence of phylogenetic constraints existing at the generic level (Kochmer and Handel 1986). In the 10 most species-rich genera of this study, such significant difference was also detected, with median flowering date ranging from middle May (*Ilex*) to late July (*Clerodendrum*). Our results implied that constraints of phylogenies might play a significant role in determining flowering patterns of phylogenetically closely related taxa (confamilials and congeners) in the DNNR. Mass flowerings within closely related taxa might benefit reproductive success via sharing floral visitors, whereas distinct flowering variation among distantly related taxa could avoid intense resource competition (Davies *et al.* 2013; Kobayashi *et al.* 2013; Liao *et al.* 2011; Numata *et al.* 2003).

Future directions

Researchers have gradually realized that data from herbarium specimen collections, in spite of their incompleteness, can offer a reliable and relatively time-saving baseline resource for examining the possible influence of abiotic and biotic factors on flowering phenological events over long time scales (Borchert 1995; Hart *et al.* 2014; Lavoie and Lachance 2006). Herbarium records facilitate own assessment on large-scale patterns of phenological responsiveness with broad species representation (Calinger *et al.* 2013). However, we should not overlook the imperfections when using flowering records from plant specimens. There were ~2.55 individuals per species (5258 individuals from 2059 species), which was probably not a sufficient data set and might bias the observed flowering pattern. Fortunately, it could be acceptable for community-level studies, particularly with the volume of herbarium voucher specimens growing with years (Panchen *et al.* 2012). Large datasets from heterogeneous sites (regional and/or global scales) can be used as a novel method to detect flowering phenological shifts among the plant communities across the spatial scale (Park 2014). Besides a herbarium-based approach, future efforts should add more flowering data in the form of onsite monitoring, including even representative intraspecific taxa (Chang-Yang *et al.* 2013; Zohner and Renner 2014).

Another aspect to be explored could be the deeper correlation analyses of flowering patterns at the community level with multiple local climatic factors from long-term forest observation stations. Unlike plant specimen collections, which have been gathered for more than 80 years, incomplete climatic records in the DNNR further limit our analyses. Flowering spectrum can be influenced by abiotic factors (e.g. temperature, sunshine duration and soil moisture content, etc.) and/or biotic factors (e.g. shared pollinators, biological competition and phylogenetic constraints, etc.) (Craine *et al.* 2012; Munguía-Rosas *et al.* 2011). An improved approach combining observed and recorded data of abiotic and biotic factors will strengthen our understanding of potential processes determining reproductive traits such as flowering phenology in forest communities.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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REFERENCES

- Ackerly DD, Cornwell WK (2007) A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. *Ecol Lett* **10**:135–45.
- APG III (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* **161**:105–21.
- Borchert R (1995) Phenology and flowering periodicity of Neotropical dry forest species: evidence from herbarium collections. *J Trop Ecol* **12**:65–80.
- Calinger KM, Queenborough S, Curtis PS (2013) Herbarium specimens reveal the footprint of climate change on flowering trends across north-central North America. *Ecol Lett* **16**:1037–44.
- Chang-Yang C-H, Lu C-L, Sun I-F, *et al.* (2013). Flowering and fruiting patterns in a subtropical rain forest, Taiwan. *Biotropica* **45**:165–74.
- Chazdon RL, Careaga S, Webb C, *et al.* (2003). Community and phylogenetic structure of reproductive traits of woody species in wet tropical forests. *Ecol Monogr* **73**:331–48.
- Clarke HL (1893) The philosophy of flower seasons. *Am Nat* **27**:769–81.
- Corlett RT (1993) Reproductive phenology of Hong Kong shrubland. *J Trop Ecol* **9**:501–10.
- Craine JM, Wolkovich EM, Towne EG (2012) The roles of shifting and filtering in generating community-level flowering phenology. *Ecography* **35**:1033–8.
- Davies TJ, Wolkovich EM, Kraft NJB, *et al.* (2013). Phylogenetic conservatism in plant phenology. *J Ecol* **101**:1520–30.
- Díez JM, Ibáñez I, Miller-Rushing AJ, *et al.* (2012) Forecasting phenology: from species variability to community patterns. *Ecol Lett* **15**:545–53.
- Elzinga JA, Atlán A, Biere A, *et al.* (2007) Time after time: flowering phenology and biotic interactions. *Trends Ecol Evol* **22**:432–9.
- Fitter AH, Fitter RS (2002) Rapid changes in flowering time in British plants. *Science* **296**:1689–91.
- Frankie G, Baker H, Opler P (1974) Comparative phenology studies of trees in tropical wet and dry forests in the lowlands of Costa Rica. *J Ecol* **62**:881–919.
- Galloway LF, Burgess KS (2012) Artificial selection on flowering time: influence on reproductive phenology across natural light environments. *J Ecol* **100**:852–61.
- Giuliani AL, Kelly EF, Knapp AK (2014) Geographic variation in growth and phenology of two dominant central US grasses: consequences for climate change. *J Plant Ecol* **7**:211–21.
- Hamann A (2004) Flowering and fruiting phenology of a Philippine submontane rain forest: climate factors as proximate and ultimate causes. *J Ecol* **92**:24–31.
- Hart R, Salick J, Ranjitkar S, *et al.* (2014) Herbarium specimens show contrasting phenological responses to Himalayan climate. *Proc Natl Acad Sci USA* **111**:10615–9.
- Körner C, Basler D (2010) Plant science. Phenology under global warming. *Science* **327**:1461–2.
- Kang H, Jang J (2004) Flowering patterns among angiosperm species in Korea: diversity and constraints. *J Plant Biol* **47**:348–55.
- Kobayashi MJ, Takeuchi Y, Kenta T, *et al.* (2013) Mass flowering of the tropical tree *Shorea beccariana* was preceded by expression changes in flowering and drought-responsive genes. *Mol Ecol* **22**:4767–82.
- Kochmer JP, Handel SN (1986) Constraints and competition in the evolution of flowering phenology. *Ecol Monogr* **56**:303–25.
- Kraft NJ, Valencia R, Ackerly DD (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. *Science* **322**:580–2.
- Lavoie C, Lachance D (2006) A new herbarium-based method for reconstructing the phenology of plant species across large areas. *Am J Bot* **93**:512–6.
- Liao K, Giture RW, Guo YH, *et al.* (2011) The presence of co-flowering species facilitates reproductive success of *Pedicularis monbeigiana* (Orobanchaceae) through variation in bumble-bee foraging behaviour. *Ann Bot* **108**:877–84.
- Li XM, She DY, Zhang DY, Liao WJ (2015a) Life history trait differentiation and local adaptation in invasive populations of *Ambrosia artemisiifolia* in China. *Oecologia* **177**:669–77.
- Li XM, Zhang DY, Liao WJ (2015b) The rhythmic expression of genes controlling flowering time in southern and northern populations of invasive *Ambrosia artemisiifolia*. *J Plant Ecol* **8**:207–12.
- Miller-Rushing AJ, Primack RB (2008) Global warming and flowering times in Thoreau's Concord: a community perspective. *Ecology* **89**:332–41.
- Munguía-Rosas MA, Ollerton J, Parra-Tabla V, *et al.* (2011) Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecol Lett* **14**:511–21.
- Numata S, Yasuda M, Okuda T, *et al.* (2003) Temporal and spatial patterns of mass flowerings on the Malay Peninsula. *Am J Bot* **90**:1025–31.
- Osada N, Sugiura S, Kawamura K, *et al.* (2003) Community-level flowering phenology and fruit set: comparative study of 25 woody species in a secondary forest in Japan. *Ecol Res* **18**:711–23.
- Ovaskainen O, Skorokhodova S, Yakovleva M, *et al.* (2013) Community-level phenological response to climate change. *Proc Natl Acad Sci USA* **110**:13434–9.
- Pérez-Harguindeguy N, Díaz S, Garnier E, *et al.* (2013). New handbook for standardised measurement of plant functional traits worldwide. *Aust J Bot* **61**:167–234.
- Panchen ZA, Primack RB, Anisko T, *et al.* (2012) Herbarium specimens, photographs, and field observations show Philadelphia area plants are responding to climate change. *Am J Bot* **99**:751–6.
- Park IW (2014) Impacts of differing community composition on flowering phenology throughout warm temperate, cool temperate and xeric environments. *Global Ecol Biogeogr* **23**:789–801.
- Petanidou T, Ellis WN, Margaritis NS, *et al.* (1995) Constraints on flowering phenology in a Phryganic (East Mediterranean shrub) community. *Am J Bot* **82**:607–20.

- Proença CEB, Filer DL, Lenza E, *et al.* (2012) Phenological Predictability Index in BRAHMS: a tool for herbarium-based phenological studies. *Ecography* **35**:289–93.
- Raunkiaer C (1934) *The Life Forms of Plants and Statistical Geography*. Oxford, UK: Oxford University Press.
- Robertson C (1895) The philosophy of flower seasons, and the phae-nological relations of the entomophilous flora and the anthophilous insect fauna. *Am Nat* **29**:97–117.
- Robertson C (1924) Phenology of entomophilous flowers. *Ecology* **5**:393–407.
- Rudolf VH, Singh M (2013) Disentangling climate change effects on species interactions: effects of temperature, phenological shifts, and body size. *Oecologia* **173**:1043–52.
- Satake A, Kawagoe T, Saburi Y, *et al.* (2013) Forecasting flowering phenology under climate warming by modelling the regulatory dynamics of flowering-time genes. *Nat Commun* **4**:2303.
- Sherry RA, Zhou X, Gu S, *et al.* (2007) Divergence of reproductive phenology under climate warming. *Proc Natl Acad Sci USA* **104**:198–202.
- Smith-Ramirez C, Armesto JJ, Figueroa J (1998) Flowering, fruiting and seed germination in Chilean rain forest Myrtaceae: ecological and phylogenetic constraints. *Plant Ecol* **136**:119–31.
- Steltzer H, Post E (2009) Ecology. Seasons and life cycles. *Science* **324**:886–7.
- Swenson NG (2013) The assembly of tropical tree communities – the advances and shortcomings of phylogenetic and functional trait analyses. *Ecography* **36**:264–76.
- Tébar FJ, Gil L, Llorens L (2004) Flowering and fruiting phenology of a xerophytic shrub community from the mountain of Mallorca (Balearic islands, Spain). *Plant Ecol* **174**:295–305.
- Tewksbury JJ, Anderson JGT, Bakker JD, *et al.* (2014) Natural History's Place in Science and Society. *BioScience* **64**:300–10.
- Uriarte M, Swenson NG, Chazdon RL, *et al.* (2010) Trait similarity, shared ancestry and the structure of neighbourhood interactions in a subtropical wet forest: implications for community assembly. *Ecol Lett* **13**:1503–14.
- Walters AW, González Sagrario ML, Schindler DE (2013) Species- and community-level responses combine to drive phenology of lake phytoplankton. *Ecology* **94**:2188–94.
- Westoby M, Wright IJ (2006) Land-plant ecology on the basis of functional traits. *Trends Ecol Evol* **21**:261–8.
- Wolkovich EM, Cook BI, Allen JM, *et al.* (2012) Warming experiments underpredict plant phenological responses to climate change. *Nature* **485**:494–7.
- Wright SJ, Calderon O (1995) Phylogenetic patterns among tropical flowering phenologies. *J Ecol* **83**:937–48.
- Wu ZY (1991) The areal-types of Chinese genera of seed plants. *Acta Bot Yunnanica* **13**:1–139.
- Wu ZY, Chen XQ (2004). *Flora Reipublicae Popularis Sinicae, Tomus 1*. Beijing, China: Science Press.
- Ye WH, Cao HL, Huang ZL, *et al.* (2008). Community structure of a 20 hm² lower subtropical evergreen broadleaved forest plot in Dinghushan, China. *J Plant Ecol* (Chinese version) **32**:274–86.
- Zhou G, Wei X, Wu Y, *et al.* (2011). Quantifying the hydrological responses to climate change in an intact forested small watershed in Southern China. *Glob Change Biol* **17**:3736–46.
- Zohner CM, Renner SS (2014) Common garden comparison of the leaf-out phenology of woody species from different native climates, combined with herbarium records, forecasts long-term change. *Ecol Lett* **17**:1016–25.