PHENOLOGICAL PATTERNS OF ENDEMIC HAWAIIAN ANGIOSPERMS

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ABSTRACT

Studies in plant phenology have provided some of the best evidence of ecological responses to recent climate change. Many phenological studies have used herbarium specimen records for analyses. Phenology studies using herbarium records were reviewed in order to summarize approaches, applications, and validations to date. The lack of studies investigating tropical phenology was addressed by analyzing herbarium specimens from the Hawaiian Islands. The flowering and fruiting phenologies of 51 endemic Hawaiian angiosperms were analyzed using herbarium records from 1837-2015. Results indicated that 75% of these species have yearly flowering patterns. Species from temperate ancestral origins likely evolved to flower outside of spring and summer months, possibly to synchronize fruiting to the onset of the wet season. Shifts in flowering over the last century were associated with both temperature and rainfall. This study demonstrated that herbarium records can be a valuable resource for understanding the phenology of tropical plants.

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CHAPTER 1

HERBARIUM SPECIMENS IN THE STUDY OF PHENOLOGY; A REVIEW OF APPLICATIONS AND METHOD VALIDATIONS

Abstract

Studies in plant phenology have provided some of the best evidence for large-scale responses to recent climate change. Over the last decade, approximately thirty studies have used herbarium specimens to analyze changes of flowering phenology over time. Thirty three of these studies were reviewed in order to summarize approaches, applications, and validations to date. Reproductive plant phenology has primarily been analyzed using two summary statistics, the mean flowering day of year and first flowering day of year. Synthesis of these studies revealed mean flowering day is a more robust statistic than first flowering. These studies used three types of regression models in analyses; flowering day on year, flowering day on temperature, and temperature on year. Most of these studies analyzed the effect of temperature by averaging temperatures from three months prior to the date of flowering. Geospatial grid data is increasingly being used for determining average temperatures at herbarium specimen collection locations. Inferences from herbarium specimen data have been shown to be comparable to findings from field data in multiple studies. Herbarium specimens are expected to become an increasingly important resource for analyzing responses to climate change. As temperatures continue to rise globally, there is a need to confirm phenological rates of change in response to warming, especially in tropical environments where phenological studies are lacking.

Introduction

Carl Linnaeus pioneered the study of phenology when he outlined methods for investigating the association between flowering and climate (Puppi 2007). The word "phenology" originates from botanist Charles Morren who introduced the term around 1850 to describe his observational studies of yearly flowering (Demarée 2009). Early field studies of plant phenology have been thoroughly reviewed by van Schaik *et al.* (1993), Fenner (1998) and Forrest et al. (2010). Long-term field observations have provided a valuable resource for analyzing phenological responses to climate change (Walther et al. 2002; Parmesan & Yohe 2003). A growing need for historical data that allows for the exploration of ecological implications of climate change has prompted researchers to look to herbarium specimens as a resource of long-term flowering data. The first study to use herbarium specimens to understand phenological responses to climate change was published by Primack *et al.* (2004). A few phenology studies such as Borchert *et al.* (1996) and Rivera & Borchert (2001) used herbarium specimens to study flowering periodicity, but not in the context of climate change. Primack *et al.* (2004) used 372 specimen records (1885-2002) and found peak flowering had advanced approximately 8 days over the last century. The Primack *et al.* (2004) publication noted that the method of using herbarium specimens may be useful for plants with either short flowering durations or long flowering durations and for plants from unique ecosystems such as mountain peaks or islands. Between 2004 and 2017, approximately 30 more studies were published using herbarium specimens. An early criticism of the method was that plants might not have been collected during their peak flowering season, potentially biasing interpretations (Lamoureux 1972). In response, authors later found that large sample sizes afforded by herbarium specimens, and the use of mean flowering times, should overcome such biases (Primack 2004; Bertin 2015). Additional criticisms of collector bias and

plant size choice were also found to be overcome by appropriate statistical analyses, especially when mean flowering times were used rather than first flowering (Robbirt *et al.* 2011; Davis *et al.* 2015).

The most common approach found in studies using herbarium specimens follows the model set by Primack *et al.* (2004). This approach can be summarized as the process of collecting flowering dates from herbarium specimens, collecting long-term temperature data from an independent source, and the use of regression analyses to analyze correlations and rates of change over time (Primack et al. 2004; Miller-Rushing et al. 2006; Gallagher et al. 2009; Robbirt et al. 2011; Gaira et al. 2011; Molnar et al. 2012; Panchen et al. 2012; Park 2012; Primack and Miller-rushing 2012; Li et al. 2013; Calinger et al. 2013; Hart et al. 2014; Rawal et al. 2015; Park & Schwartz 2015). Linear regression models are the most widely used statistical models in field studies investigating flowering phenology (Zhao et al. 2013). Primack *et al.* (2004) used three types of linear regression models that were later applied to other studies of phenology based on herbarium specimen data (Table 1). Primack *et al.* (2004) regressed flowering day on temperature, flowering day on year, and temperature on year. Results from these models address whether flowering day was earlier in the year when temperatures were warmer, whether flowering day was earlier in the year over time, and whether temperatures were warmer over time. About 30% of the studies in this review used all three types of regression models in their analyses (Table 1). Approximately 82% modeled flowering day on year to show long-term changes in flowering (Table 1). About 64% modeled the effect of temperature on flowering day and a different 64% modeled long-term changes in temperature (Table 1).

These studies have primarily been conducted with specimens from herbaria in temperate regions such as the Eastern Himalayas (Gaira *et al.* 2011; Li *et al.* 2013; Gaira *et al.* 2014; Hart

et al. 2014), Southern Australia (Gallagher *et al.*, 2009; 2012; Rawal *et al.*, 2015), Northern Europe (Robbirt *et al.* 2011; Diskin *et al.* 2012; Molnár V *et al.* 2012), and North America (Primack *et al.* 2004; Lavoie and Lachance 2006; Miller-Rushing *et al.* 2006; Primack 2009; Neil *et al.* 2010; Panchen *et al.* 2012; Park 2012; Primack and Miller-rushing 2012; Searcy 2012; Calinger *et al.* 2013; Park 2014; Park & Schwartz 2015; Bertin 2015; Davis *et al.* 2015). Although studies by Borchert (1996) and Zalamea et al. (2011) analyzed flowering periodicity in tropical plants using herbarium specimens, I found no study to date that has used herbarium specimens to analyze the effect of recent climate change in a tropical region. This review examines how studies chose sample sizes, flowering specimens, temperature averages and geographical scale in their analyses. This review also examines how these studies validated the use of herbarium specimens and provides suggestions for methods to be used in future studies.

Review

Sample size

Sample size, or the number of specimens used per species, varied across studies (Table 1). The minimum number of specimens used per species was occasionally as low as two or three records (Searcy 2012). Low sample sizes prompted Bertin (2015) to provide a detailed analysis of how sample size affected mean, median, range, early flowering and late flowering summary statistics. In random simulations comparing sample sizes, mean flowering day values deviated less than 5 days for species with as few as 4 samples (Bertin 2015). Bertin (2015) concluded that the mean was a more accurate estimator of phenology than other estimators of early flowering. Bertin (2015) also showed that by increasing the sample size to 20, mean flowering times deviated only 1-2 days. Similarly, a study by Miller-Rushing & Primack (2008) used field data and found that small sample sizes led to biased estimators of first-flowering dates, but mean flowering day was

not biased by sample sizes. Moussus *et al.* (2010) investigated sample sizes by simulating 10 known phenological estimators such as mean flowering day and first-flowering date. After comparing known phenological shifts with shift estimations from models using the same data, Moussus *et al.* (2010) concluded that first-flowering dates were inaccurate and showed much a greater bias than mean flowering day.

Other studies using herbarium data have set a minimum number of herbarium specimen samples per species to more accurately estimate phenology. Calinger *et al.* (2013) and Gallagher *et al.* (2009) set a minimum of 10 specimens in order to meet statistical assumptions of different models. Molnar *et al.* (2012) eliminated a species from analyses because collections only yielded dates across an 8 year time span and Park & Schwartz (2015) eliminated species with records that spanned less than 3 years. A study by Neil *et al.* (2009) organized species into functional groups (spring ephemerals, spring shrubs, fall ephemerals, winter-spring ephemerals, and winter-spring shrubs) in order to overcome the problem of low sample size for each species. Responses of individual species within these functional groups varied greatly however and the aggregated data showed limited significant results. In order to analyze species distributions using herbarium specimens, van Proosdij *et al.* (2016) found that the minimum number of herbarium specimen samples should be between 14 and 25 depending on the geographical range of the species. The van Proosdij *et al.* (2016) study used simulated species to assess the minimum herbarium samples required for acceptable model performance in both virtual and real study areas. Some species with narrow geographical ranges could be modeled with as few as 14 herbarium records while wide ranging species could be satisfactorily modeled with a minimum of 25 records (van Proosdij *et al.* 2016). Based on these studies, I recommend caution when interpreting results from samples sizes with fewer than 30 records (Miller-Rushing *et al.* 2008;

Moussus *et al.* 2010; Bertin 2015). The average sample size across studies in this review was about 55 samples per species (Table 1.). I also recommend using a minimum of 10 herbarium records per species when conducting regression analyses and the mean day of year should be used rather than first flowering (Calinger *et al.* 2013; Gallagher *et al.* 2009).

Determining flowering status of specimens

Studies often examined each individual specimen and recorded the presence or absence of flowers. Some studies used more detailed criteria to assess flowering specimens. Diskin *et al.* (2011) used a scoring system to categorize the stages of flowering on each specimen. Calinger *et al.* (2013) only used specimens with more than 50% of flowers in anthesis to help ensure that the samples were in peak flowering. For a species with an inflorescence, Davis *et al.* (2015) only counted specimens as "flowering" if greater than 75% of flowers were open.

Haggerty *et al.* (2012) provided a primer to help phenology researchers collect data from herbarium specimens. Haggerty *et al.* (2012) stated that researchers must assume the stem on the herbarium sheet represents the flowering phenophase for the entire plant. Early on, Lamoureux (1972) noted that he would not accept this assumption and phenophase variation of individual plants became his reason for not using herbarium specimens to investigate plant phenology in the Hawaiian Islands. Phenological studies in tropical regions have similar research challenges as studies in temperate regions, such as long flowering durations, geographical climate variation, and genetic variation (van Schaik 1993; Fenner 1998).

Studies in temperate regions have used varying methods to account for long flowering durations. For example, Molnar *et al.* (2012) and Bertin (2015) excluded species that flowered outside of the peak flowering season, defined as the period from late-spring to early-summer.

Molnar *et al.* (2012) removed a species because its peak flowering date was in September and focused on 40 other taxa that had flowering peaks from late-spring to early-summer. The excluded species was a strong outlier and it was suggested that autumn climate events might affect species differently than spring climate events (Molnar *et al.*, 2012). Park (2012) also removed outlier records when flowering records fell outside the peak flowering season. Flowering records before day 45 and after day 310 on the 365 day year were removed from analyses to reduce biases caused by winter flowering species. Additionally Park (2012) removed records of flowering that were 150 days after the median flowering date for each species to reduce errors caused by any second flowerings that happen in autumn months.

Several other studies removed taxa with long flowering durations to reduce variance among species. Bertin (2015) excluded native weedy species with flowering durations from spring to fall. Gallagher et al. (2009) only used species that flowered less than 3 months. Panchen et al. (2012) chose to use only species with clear beginning and ending points to investigate long and short flowering duration. Panchen et al. (2012) found that plants with shorter flowering durations required smaller sample sizes to produce significant results when regressing flowering day on year. Other studies such as Calinger *et al.* (2013) and Lavoie & Lachance (2006) disregarded the effect of flowering duration and noted the results of Primack *et al.* (2004), which found that there was no bias associated with long or short flowering durations when mean estimations are analyzed. While flowering durations were adjusted in some studies, most studies conducted analyses using all flowering dates, and this will likely be the trend for analyses in the future. If records are removed at all, it may be best to only remove records from the far tail ends of the flowering day distribution of individual species, rather than removing records that are outside the peak-flowering season of a community.

Averaging temperatures

The foundational study by Primack et al. (2004) used temperature averages from three calendar months prior to the specimen flowering date. Field investigations such as Fitter et al. (1995) have shown temperature averages from different sets of months preceding flowering affect flowering phenology in different ways. Calinger *et al.* (2013) chose to regress the month of flowering with temperature averages from each of the 11 months prior to flowering. They found that temperatures averages from three months prior to the date of flowering showed the strongest correlations with flowering (Calinger *et al.* 2013). A study by Robbirt *et al.* (2011) investigated temperature averages for three sets of temperature averages over three month long intervals and also found that three months prior to flowering had the most predictive power. Similarly, Rawal *et al.* (2015) used temperature averages from 1, 3, 6, 9, and 12 months prior to flowering because responses often vary by species. Rawal et al. (2015) also found that mean temperatures three months prior had the greatest influence on flowering time.

Other studies have used average temperatures from spring months because spring temperatures have shown the most predictive power for flowering (Miller-Rushing & Primack 2008; Primack et al. 2009; Robbirt *et al.* 2011; Calinger *et al.* 2013; Park 2014; Park & Schwartz 2015). Bertin (2015) found an interesting trend that supported the effect of spring temperatures. Bertin (2015) found that the earlier a species mean flowering time occurred in the spring, the more the species mean dates had shifted toward an earlier day of year over time. Robbirt *et al.* (2011) also found the highest correlations from spring temperature averages across March, April and May. Gaira *et al.* (2011) found the highest correlations in the months of December-February. Calinger *et al.* (2013) found significant changes in flowering in response to average spring temperatures (February-May) but not in response to summer temperatures (June to

September). Alternatively, Diskin *et al.* (2011) investigated the averages of temperature anomalies, or deviations from the overall long-term mean, for two, three and six month periods from January to June and found the averages from six months prior to flowering had the strongest correlations.

Park (2014) used temperature averages across three one-month periods from early spring to late summer and found a similar trend. Temperature averages were organized into early, mid, and late seasonal classes within the months of February to October. Park (2014) found warming temperatures had affected species in the early spring class more than other classes. Park & Schwartz (2015) also used early, mid and late seasonal classes for spring and summer and found that mid-season phenology events should be modeled differently than early or late season events.

Hart *et al.* (2014) used annual temperatures and temperatures from each season (spring, summer, fall, and winter) and found significant correlations for annual and fall temperature averages, but with opposite effects. Hart et al. (2014) discussed that warmer fall temperatures may delay the chilling requirement for *Rhododendron* species and hence delay flowering while warmer annual temperatures will show advances in flowering overall.

Other studies found annual temperature means were as useful as spring temperatures. Davis *et al.* (2015) found similar results between spring and annual temperature averages and used annual averages in analyses. Gallagher *et al.* (2009) also used annual temperature means for analyses and explained that seasonal means were correlated with annual means.

I recommend investigating the effect of temperature by analyzing averages from multiple sets of months prior to flowering for each species rather than using only spring or only annual temperatures (Diskin *et al.* 2011; Robbirt *et al.* 2011; Calinger *et al.* 2013). Caution should be taken when analyzing temperature averages from the same number of months prior to flowering

for all species. For example, when analyzing the effect of temperature averages from 3 months prior for all species, Calinger *et al.* (2013) found that species with a mean flowering day in April were not strongly correlated with January temperatures, therefore temperature averages from the months of February, March and April were used instead for those specific species. Therefore, it may be important to use different sets of temperature averages for different species.

Geographic variation

Several methods have been used to account for climate variability across a species' range. An early study by Lavoie & Lachance (2006) investigated the effects of climate variation on the phenology of Coltsfoot (*Tussilago farfara* L.) across a range of about 10,000 km^2 in Quebec, Canada. Temperature data from 88 meteorological stations were averaged together across this range. To account for early snow cover melt in the southern part of this range, flowering dates from individuals in southern locations were normalized with individuals in northern locations by subtracting extra periods of snow cover from individuals in the north. The adjusted dates indicated flowering occurred 33 days earlier over the last century while original (unadjusted) dates indicated flowering occurred 19 days earlier over the last century.

While the study by Lavoie & Lachance (2006) adjusted actual dates for analyses, more recent studies mostly account for climate variation using geographical divisions of various scales. Calinger *et al.* (2013) accounted for climate variation across Ohio by using temperature averages from 10 US Climate Divisions across the state, each about 8,000 km^2. A total of 344 Climatic Divisions were established across the contiguous United States in 1895 in order to monitor climate records more accurately. These divisions have now accumulated about 100 years of climate records (Guttman & Quayle 1996). A later study by Park (2014) used average temperatures across the U.S. county where each specimen was collected.

Other studies accounted for climate variation across longitude, latitude, or elevation. Robbirt *et al.* (2011) analyzed the geographical effect of longitude and found that flowering occurred 4.86 days earlier per degree of longitude in a westward direction across the southern coastal counties of England (Robbirt *et al.* 2011). A later study by Bertin (2015) used Hopkins' bioclimatic law to normalize dates on specimens. Hopkins' (1918) generally stated that for every increase in a degree of latitude, or increase of 121.92 m elevation, the life history events of plants and animals were delayed by 4 days. Bertin (2015) found consistencies with Hopkins' bioclimatic law using latitude and elevation and chose to normalize flowering dates by adding expected phenological deviations from both latitude and elevation. Gaira *et al.* (2011) also analyzed climate variation using elevation when temperature data were not available assuming a 6.5°C change in temperature per 1000 m change in elevation in the Himalayan region.

Other studies used temperature averages across large regions. Li *et al.* (2013) used temperature data that was averaged from 36 meteorological stations across the Tibet Autonomous Region. Molnar *et al.* (2012) used temperature averages from 10 meteorological stations across Hungary and stated that the data were statistically indistinguishable across stations (~93,030 km²). Park & Schwartz (2015) averaged temperatures from 13 stations across South Carolina, USA (~82, 931 km²). A later study by Robbirt *et al.* (2014) used temperature averages from an area between Bristol, Preston, and London, across the United Kingdom (~17, 000 km²). Robbirt *et al.* (2014) used geographical divisions called Watsonian vice-counties specifically delineated for the purpose of collecting scientific data, much like the US Climate Divisions. Robbirt *et al.* (2014) found temperature averages were sufficient because climate variation across the Watsonian vice-counties used in their study did not significantly differ.

In order to more accurately determine temperature averages across a region, recent studies have used Geographical Information System (GIS) software to extract temperature data from specific Global Positioning System (GPS) points. A study by Gallagher *et al.* (2009) referenced GPS locations for each specimen and extracted the temperature averages at specimen GPS points from a gridded map of temperature averages across Australia (~5 km^2 resolution). A study by Rawal *et al.* (2015) also used the nearest data point from gridded climate averages across Victoria, Australia. A recent study by Edward & Still (2008) analyzed the climate envelopes of grasses by assigning GPS points to herbarium specimen locations in order to extract temperature averages from gridded climate maps (250m^2 resolution). Studies using GPS data are able to account for climate variation with higher resolution, although accuracy still depends on the underlying empirical data and modeling approach used to generate GIS climate layers.

I recommend using the most precise temperature data available, such as climate divisions (Calinger *et al.* 2013; Robbirt *et al.* 2015) rather than state or region averages (Li *et al.* 2013; Park & Schwartz 2015). Using GPS specimen data to identify local climate conditions from GIS climate layers (Gallagher *et al.* 2009; Edward & Still 2008) is also now generally more precise and convenient to use than making generic and coarse-scale corrections for latitude, longitude or elevation (Gaira et al. 2011; Robbirt *et al.* 2011; Bertin 2015). If temperature averages from larger areas are used, I recommend testing for the effect of climate variability across smaller divisions before using averages across the larger area (Lavoie & Lachange 2006; Molnar *et al.* 2012; Robbirt *et al.* 2015).

Validations

Field data are often combined with herbarium specimen data in analyses (Primack *et al.*, 2004; Miller-Rushing *et al.* 2006; Bertin 2015). Primack *et al.* (2004) used herbarium specimens to

find a “historic” flowering mean and then used field data to find a “current” flowering mean (Primack *et al.*, 2004). The historic mean derived from specimens was compared to the current mean from field data in order to determine if there was a statistical difference in mean flowering between the two time periods. Primack *et al.* (2004) found that the flowering duration of each species was statistically indistinguishable between herbarium and field data and therefore concluded that herbarium and field data were compatible. Studies by Miller-Rushing *et al.* (2006) and Bertin (2015) also compared historical averages with current averages using specimens for historical data and field samples for current data. Miller-Rushing *et al.* (2006) found that results from herbarium specimens alone differed from the combined data by about one day.

An early study by Borchert, (1996) found that using herbarium specimen data produced slightly longer flowering durations than field data, but noted that durations were mostly similar overall. In Borchert, (1996) and in Rivera & Borchert (2001), phenology data from a field sites largely overlapped that of herbarium specimen findings with only slight differences. The negligible differences between herbarium specimen data and field data in these studies helped justify the use of specimen data in more recent studies.

Bolmgren & Lonnberg (2005) tested the validity of using specimen data specifically. Bolmgren & Lonnberg (2005) compared specimen data directly to field data and found the two data sets were overall highly correlated with only minor differences. For example, herbarium specimens showed a slightly earlier mean flowering for spring-flowering plants than field data but the difference was not significant (Bolmgren & Lonnberg, 2005). Later studies by Robbirt *et al.* (2011) and Davis *et al.* (2015) also primarily focused on testing the validity of using herbarium specimen data. Robbirt *et al.* (2011) used a principal axis regression analysis to

compare herbarium derived peak-flowering dates with field derived peak-flowering dates and found a high degree of correlation. Robbirt *et al.* (2011) discusses how the high degree of correlation between herbarium and field data also supports the notion that geographically different records will not significantly alter the robustness of either data set. A study by Davis *et al.* (2015) used a paired *t* test to compare the mean of first flowering day between specimen and field data and found no statistical difference. Davis *et al.* (2015) concluded that both specimen and field data could be combined and used as a whole.

In order to increase sample sizes, Molnar *et al.* (2012) added about 2000 field observations to about 5000 herbarium records, totaling 70% herbarium records for the study. Similarly, Panchen (2012) added about 2000 field records to about 1500 herbarium records, for a total of 43% herbarium records for the study. Searcy (2012) combined specimen and field data and then split the combined data into two time periods (1863–1935 and 1994–2008). Despite criticisms, herbarium specimen data have been shown to produce similar enough results to field data that herbarium specimen data are now widely accepted in phenological studies.

Conclusions

The use of herbarium specimens for the investigation of flowering phenology has grown considerably during the past decade. As efforts to produce digital copies of specimens and label information have amassed large datasets, new approaches for analyzing responses to climate change are becoming available. Studies using herbarium specimens have become an asset for long-term climate change vulnerability assessment. Studies using herbarium specimens have also begun to analyze the effects of climate change associated with community composition (Miller-Rushing & Primack 2008, Park 2014) coevolved plant pollinator relationships (Molnar *et*

al. 2012; Robbirt *et al.* 2014) functional groups (Miller-Rushing & Primack 2008; Panchen et al 2012; Calinger *et al.* 2013) and phylogenetic relationships (Bolmgren & Lonnberg 2005; Molnar *et al.* 2012; Primack and Miller-rushing 2012).

While studies using specimen data to analyze long-term changes have been limited to temperate regions, future studies could use circular statistics to analyzing the long-term phenological changes in tropical regions. Circular statistics have been used to analyze flowering phenology in several tropical field studies but these studies lack long-term climate change analyses (Novotny & Basset 1998; Morellato et al. 2000; Cruz et al. 2006; Rogerio & Araujo 2010; Tesfaye et al. 2011; Nadia et al. 2012; Nazareno & dos Reis 2012; Staggemeier et al. 2012; Carvalho & Sartori 2015; Kebede & Isotalo 2016). Although small sample sizes have been used in early studies of phenology, various factors, such as long flowering duration or wide geographic range, may require larger sample sizes. Based on recent validations, mean estimations of peak flowering should be used rather than first flowering because first flowering is often inaccurate. The use of GPS data appears to be the way forward for the advancement of methods in the study of phenology. GPS point data will allow for the collection of higher resolution temperature averages across diverse geographical regions. Furthermore, GPS data will become the standard for phenology studies in tropical and montane regions that are difficult to access. Studies using herbarium specimen data will continue to help us understand the impact of recent climate change on plant reproductive phenology. Future studies in phenological research using herbarium specimens are recommended to focus on the importance of phylogenetic signals and plasticity in order to further improve our understanding of adaptation and resilience to climate change.

Table 1. Methods of studies; The column "Flw Day ~ Temp" represents studies that conducted a type of regression analysis with flowering day (Flw Day) as the dependent variable and temperature average (Temp) as the independent variable. This follows for columns using a tilde (~) which include the independent variable "Year". The "$\Delta \bar{x}$" symbol represents studies that analyzed a difference in the mean flowering day between Historic and Current time period groups rather than using a type of regression analysis.

species	specimens	authors	year	specimens /species	Flw Day ~ Temp	Flw Day ~ Year	Temp ~ Year
1	117	Gaira et al.	2011	117		x	x
1	NA	Gaira et al.	2014	NA	x	x	x
1	192	Robbirt et al.	2011	192	x		
5	158	Rawal et al.	2015	32	x	x	
5	540	Diskin et al.	2012	108	x	x	x
20	371	Gallagher et al.	2009	19	x	x	x
20	1108	Davis et al.	2015	55	x	x	x
28	1587	Panchen et al.	2012	57	x	x	
36	460	Hart et al.	2014	13	x		x
>37	372	Primack et al.	2004	10	x	x	x
39	216	Lavoie & lachange	2006	6		x	x
39	5424	Molnár et al.	2012	139		x	x
41	909	Li et al.	2013	22	x	x	x
42	142	Miller-Rushing et al.	2006	3	x	x	x
43	NA	Primack & Miller-Rushing	2012	NA		x	x
87	NA	Neil et al.	2010	NA		x	
141	5053	Calinger et al.	2013	36	x		x
186	30,000	Bertin	2015	161		$\Delta \bar{x}$	x
370	1125	Searcy	2012	3		$\Delta \bar{x}$	
1185	5949	Park	2012	5		x	
>1700	19,328	Park	2014	11	x		
24,105	823,033	Park & Schwartz	2015	34	x	x	

CHAPTER 2

FROM ANCESTRAL ORIGINS TO SEASONAL ADAPTATIONS; HERBARIUM SPECIMENS REVEAL PHENOLOGICAL PATTERNS IN HAWAIIAN PLANTS

Plant phenology of the Hawaiian flora is largely unknown or lacks long-term quantitative investigation. This study investigated flowering and fruiting patterns of 51 endemic Hawaiian angiosperms using 5,517 herbarium records from 1837-2015. Tropical and temperate ancestral biogeographic origin was investigated as a potential predictor of phenology. Time from flowering to fruiting was also investigated as a predictor of flowering season. Results indicated that 75% of these species showed significantly non-uniform flowering patterns and 55% showed significantly non-uniform fruiting patterns. Most species had a mean flowering day of year in July when daylength is longest and most species had a mean fruiting day of year in August around the onset of the wet season. Several congeneric species pairs showed a flowering mean within about 5 days of each other while other congeneric species' flowering means were different by as much as 100 days. Since these congeners speciated from the same colonization events and are mostly sympatric, mechanisms structuring these inconsistent phylogenetic patterns of phenology are not well understood. Plants with temperate ancestral biogeography did not show an overall significantly different flowering phenology from plants with tropical ancestral biogeography. Length of time from flowering to fruiting was negatively correlated with flowering day of year such that species with longer maturation periods flowered earlier in the year. This investigation revealed that some species from temperate ancestral origins evolved to flower outside of spring and summer months, possibly to synchronize fruiting to the onset of the wet season.

Introduction

Multiple biotic and abiotic factors influence plant reproductive phenology in tropical environments (van Riper III 1980; Fenner 1998; Forrest & Miller-rushing 2010). Flowering time of year is likely related to abiotic cues such as solar radiation, temperature and precipitation, while flowering duration is likely related to biotic cues such as pollination and predation (Sakai 2001). Phenological patterns in tropical dry forests are often influenced by changes in precipitation (Borchert 1996; Fenner 1998; Brearley et al. 2007; Zimmerman et al. 2007; Forrest & Miller-rushing 2010) while patterns in wet tropical environments, such as rainforests, often synchronize their fruit maturation with the beginning of the wettest season (Howe & Smallwood 1982, Garwood 1983, Tesfaye et al. 2011; Aravind et al. 2013). Borchert et al. (2005) investigated the effect of photoperiod on phenology in tropical plants at the equator where day length is the same throughout the year. The study found tropical plants at the equator responded to as little as 4 minutes of change in the timing of the sunrise. Topical species also synchronize flowering to increase pollination success when populations are at low densities in highly diverse communities (Augspurger 1980, Borchert 2005).

Several studies have used circular statistics to analyze plant phenology in tropical environments because flowering and fruiting are observed throughout the year and flowering cues vary (Novotny & Basset 1998; Morellato et al. 2000; Cruz et al. 2006; Rogerio & Araujo 2010; Tesfaye et al. 2011; Nadia et al. 2012; Nazareno & dos Reis 2012; Staggemeier et al. 2012; Aravind et al. 2013; Carvalho & Sartori 2015; Kebede & Isotalo 2016). Morellato et al. (2000) found flowering to be correlated with both day length and temperature in the Atlantic rain forests of southeastern Brazil using circular statistics. Cruz et al. (2005) found two sympatric *Heliconia* species flowered in synchronized patterns with little overlap and slightly different

pollinators using circular statistics. Nadia et al. (2012) found precipitation to have the strongest correlation with four species in North-Eastern Brazil after using circular statistics in analyses. While precipitation is often strongly associated with phenology in tropical regions, other environmental factors also affect tropical plant phenology.

In temperate regions, several studies using herbarium records have found support for the hypothesis that long-term phenological patterns are most sensitive to changes in temperature (Park & Schwartz 2014; Davis et al. 2015; Rawal et al. 2015). Studies of long-term phenology are lacking for many tropical regions however. For example, phenological studies in the Hawaiian Islands are largely unknown or lack long-term quantitative support. Results from early quantitative field observations in the Hawaiian Islands suggest at least eight endemic species show seasonal flowering, but environmental factors for flowering were largely undetermined (Lamoureux 1973, van Riper III 1980, Berlin et al. 2000). A two year field study comparing the flowering phenology of two Hawaiian species, *Sophora chrysophylla* (Fabaceae) and *Myoporum sandwicense* (Myoporaceae), suggested the flowering of *S. chrysophylla* may have been in response to rainfall (van Riper III 1980). A more recent study investigating ten Hawaiian species over three years found that most species showed peak flowering times between May and August, when solar radiation was highest (Berlin et al. 2000). There is also evidence that fruit maturation primarily occurs in the wet season in the Hawaiian Islands. A field study by Drake (1998) investigated seed rain and seed banks on Hawaii Island and found that several native species produced the most seeds during the rainy season in the month of January. A year-long investigation by Bakutis (2005) also found seasonal peaks in fruit production during the wet season on Oahu where several endemic Hawaiian species showed a significant fruiting peak in December. As climate change continues to threaten native Hawaiian plant species, many

important questions about the extent and establishment of phenological patterns in the Hawaiian flora still remain to be addressed (Krushelnycky et al. 2013).

Phylogenetic relationships play an important role in reproductive phenology (Forrest & Miller-rushing 2010; Pau & Still 2014; Du et al. 2015). A recent study investigating phenology for over 19,000 plant species across China found support for the phylogenetic constraint hypothesis of flowering phenology, which states that closely related species flower at similar times of year (Du et al. 2015). A study by Davies et al. (2014) also found phylogenetic conservatism in flowering phenology when investigating a phylogenetic signal for phenological responses to environmental variables. Staggemeier et al. (2010) found a phylogenetic signal for flowering phenology across 34 tropical Myrtaceae species in South-Eastern Brazil. The benefits of phenological synchronization, such as pollination success for conspecific species or shared pollinators for congeneric species, are likely mechanisms driving phylogenetically conserved phenological traits.

The Hawaiian flora consists of a geographically diverse group of colonists from both temperate and tropical origins and provides a relatively recent evolutionary timeline for investigating the evolution of phenological patterns (Keeley & Funk 2011). Ancestors of endemic Hawaiian plant species colonized the archipelago as much as six million years ago from several continents including Africa, Asia, Australia and North and South America (Baldwin et al. 1991; Kim et al. 1998; Wagner et al. 1999; Ballard and Sytsma 2000; Ziegler 2002; Lindqvist and Albert 2002; Costello & Motley 2007; Harbaugh 2008; Bacon et al. 2011; Qi et al. 2013; Cantley et al. 2014; Hayward & Horton 2014; Le Roux et al. 2014). Ancestral origins have played a role in structuring biogeographic patterns of Hawaiian grasses and also may be important in structuring flowering patterns (Edwards & Still 2008).

I address the lack of long-term phenological data for endemic Hawaiian plants by analyzing flowering and fruiting using herbarium specimens and circular statistics. Morellato et al. (2010) has discussed the growing use of circular statistics in studies of tropical plant phenology. Specifically, circular statistics have been used in situations where species have long flowering durations or when peak flowering occurs near December or January (Staggemeier et al. 2010, Nadia et al. 2012, Aravind et al. 2013, Carvalho & Sartori 2015). I hypothesized that Hawaiian plant species would show non-uniform flowering and fruiting patterns because other tropical species have shown peak flowering times, despite flowering in small amounts throughout the year (Kikim & Yadava 2001; Márcia et al. 2004). I tested this hypothesis by predicting that flowering distributions would be non-uniform.

I also hypothesized that species with long period of flower to fruit maturation would flower at an earlier time of year than species with shorter flower to fruit periods because fruiting is often synchronized with the onset of the wet season in tropical environments (Howe & Smallwood 1982, Aravind et al. 2013). To address evolutionary changes in phenology, I asked whether flowering times of year were constrained by ancestral biogeographic origins. I hypothesized that species from temperate ancestral origins would show peak flowering in spring and summer months when daylength is longest. I tested this hypothesis by predicting that the circular mean flowering day of year for species of temperate ancestral origins would more likely occur in the months between March and August. Furthermore, I hypothesized that species in dry seasonal environments would be more likely to show seasonal flowering than species in consistently wet environments because plants in tropical dry environments show strong correlations with seasonal precipitation (Forrest & Miller-rushing 2010). I tested this hypothesis by predicting that Hawaiian plants occurring in wet environments would be more likely to show

uniform flowering throughout the year than Hawaiian plants in dry seasonal environments. I also hypothesized that species in the same genus were more likely to flower at the same time of year than species in different genera because flowering time of year often shows a phylogenetic signal in closely related species (Du et al. 2015).

Methods & Materials

A total of 14,050 herbarium specimens were examined for flowering at The Bernice Pauahi Bishop Museum, Hawaii (BISH) from October 2015 to January 2016. I examined each specimen for the presence and absence of flowers or fruits. I also recorded flowering for each specimen in groups categorized by one open flower, five or more open flowers, many flowers, or remnant flowers. I recorded fruiting for each specimen in groups categorized by immature fruits, one fruit, five or more fruits, many fruits, and remnant fruits. My final analyses considered a specimen as flowering or fruiting if it fell into any of the above categories except for remnant flowers or remnant fruits, respectively. Remnant flowers were largely hardened calyx vascular tissue that remained on the individual plant after other dermal and ground tissues were removed by weathered in the field before being collected. This was especially observed for species such as for *Kadua centranthoides*. I had observed these persistent remnant flower parts on plants in the field for several species before viewing specimens, and their presence was not indicative of flowering. Immature fruits were categorized as fruits that were less than about 75% of their mature size. Remnant fruits mainly included seedpods and capsules that had dehisced and contorted with one or no withered seeds remaining, or a very small fruit that appeared to have not fully developed. Immature fruits mainly included very small fruits that, in some cases, began to develop while the flower corolla was still intact. This was observed for species such as

Sophora chrysophylla.

Species name, collection date and collection location was recorded from each specimen label with flowers or fruits. Dates from duplicate specimen collections were removed from analyses (Rawal et al. 2015). After removing duplicate specimen dates, a final count of 5,517 flowering and 6,164 fruiting records remained. These included a total of 51 species from 24 families collected between 1837 and 2015.

The mean flowering day of year and the distribution of flowering and fruiting for each species was analyzed using circular statistics. The calendar day (1 - 365) was collected from each specimen collection date and then converted into an angular variable (1° - 360°) for flowering and fruiting day of year analyses. The Rayleigh test was conducted to test for non-uniformity in the distribution of flowering days for each species with a null hypothesis that the distribution was not different from a uniform distribution and an alternative hypothesis that the distribution was not uniform (usually unimodal by visual inspection).

Estimated maturation time was defined as the angular distance from the mean flowering day of year to the mean fruiting day of year. I tested the hypothesis that maturation time was negatively correlated with mean flowering day of year, such that, fruiting was synchronized to the onset of the wet season. To analyze whether geographical ancestral origin was independent of the mean flowering day, I assigned each species to a "temperate" or "tropical" origin based on available phylogenetic and biogeographic literature. I also assigned each species to "spring/summer" or "fall/winter" flowering seasons using the mean flowering day of year for the species. I conducted a Fisher's Exact Test to determine if ancestral origin was independent of flowering season.

A Fisher's Exact Test was also conducted to determine whether species in seasonally dry

environments were more likely to have a non-uniform distribution (seasonal flowering) than species in consistently wet environments. I assigned 21 species to the "dry" climate zone and 30 species to the "wet" climate zone. In order to define climate zones I collected annual rainfall averages across the geographical range of each species using specimen locations and gridded raster layers of annual rainfall in Hawaii (Frazier et al. 2016) using ArcGIS 10.3.1 (USA Topo Maps, National Geographic Society 2013). Geographical areas that receive more than 2280 mm of average annual rainfall also receive more consistent rainfall throughout the year (Rainfall Atlas of Hawaii, Giambelluca et al. 2008), therefore, I assigned species with less than 2280 mm of average annual rainfall to the "dry" seasonal zone and the remaining species were assigned to the "wet" zone.

To analyze whether species in the same genus flowered at a more similar time of year than species in different genera, I found the angular difference in mean flowering day of year between pairs of species in the same genus and between species in a random set of different genera. A Watson-Wheeler Test for homogeneity of means for circular data was conducted to test the null hypothesis that flowering mean differences between species in the same genus were indistinguishable from differences between species of different genera. Analyses were conducted using the package Circular of the statistical software R (R Development Core Team 2016). The software Oriana 4.0 (Kovach Computing Services) was also used for data management and visualization.

Results

Flowering patterns for 38 of the 51 species (75%) were significantly non-uniform (Rayleigh test, $p < 0.05$; Table 2) and a total of 28 of the 51 species (55%) showed fruiting patterns that were

significantly non-uniform (Rayleigh test, $p < 0.05$; Table 2). For many species, I found specimens categorized a having "many flowers" or "many fruits" were also collected at about the mean flowering or fruiting day of year. This indicated that the mean flowering day of year was also likely the peak flowering time for most species. Mean flowering day occurred during July (27%) more than any other month and mean fruiting occurred in August (24%) more than any other month (Fig. 1).

When I included specimens without flowers or fruits in analyses, I found uniform distributions for most species. This indicated that collection bias was not likely to affect results because collections happened through the year. Of the 24 different plant families represented, Rubiaceae contributed the most species with 11 taxa (21.1%), in 4 genera. The remaining plant families contributed between 1 and 3 species.

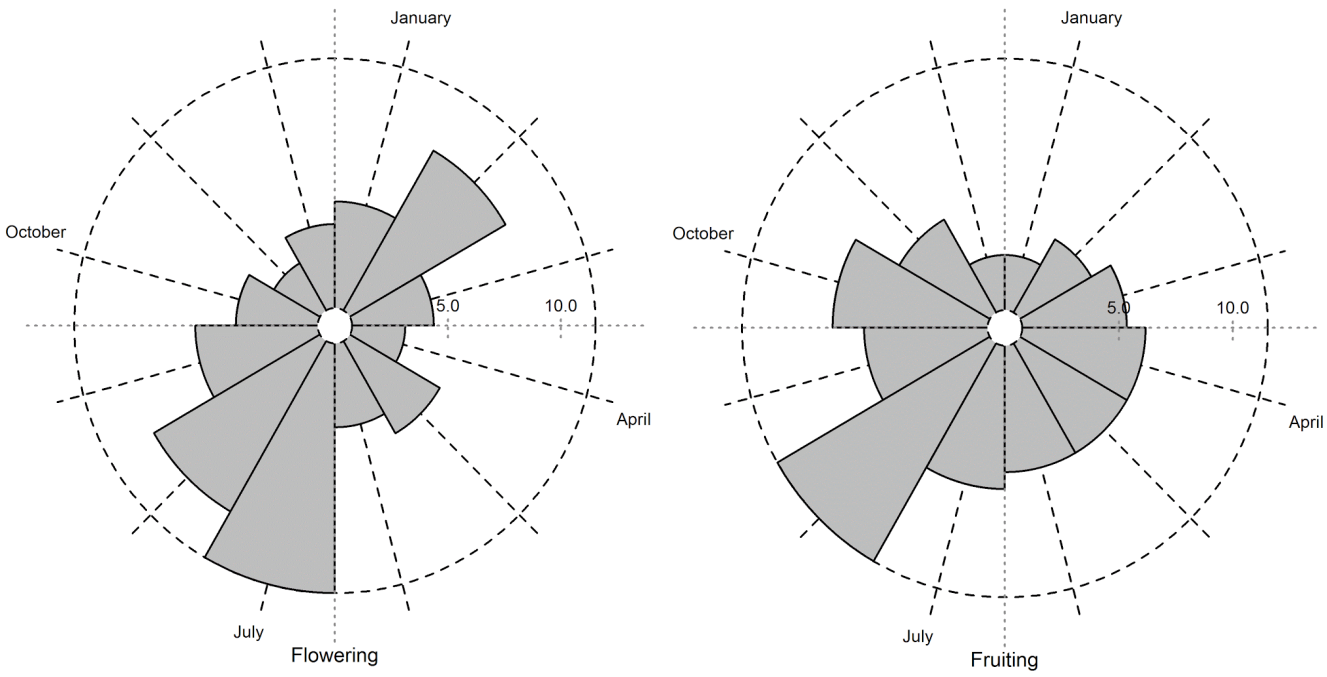


Figure 1. Windrose plots of all species mean flowering day (left) and mean fruiting day (right). More species flowered in July than any other month and more species showed fruiting in August more than any other month. Each petal (bin) is the frequencies of species with a mean in that month and the axes indicate the frequency count.

A total of 24 species showed both; 1) a non-uniform flowering and, 2) a non-uniform fruiting distribution. These 24 species were used to find estimated maturation time periods. Estimated maturation time periods ranged from about 1 to 173 days. A significant correlation was found between the mean flowering day of year and maturation time (Fig. 2, $r = .577$, $p < .001$).

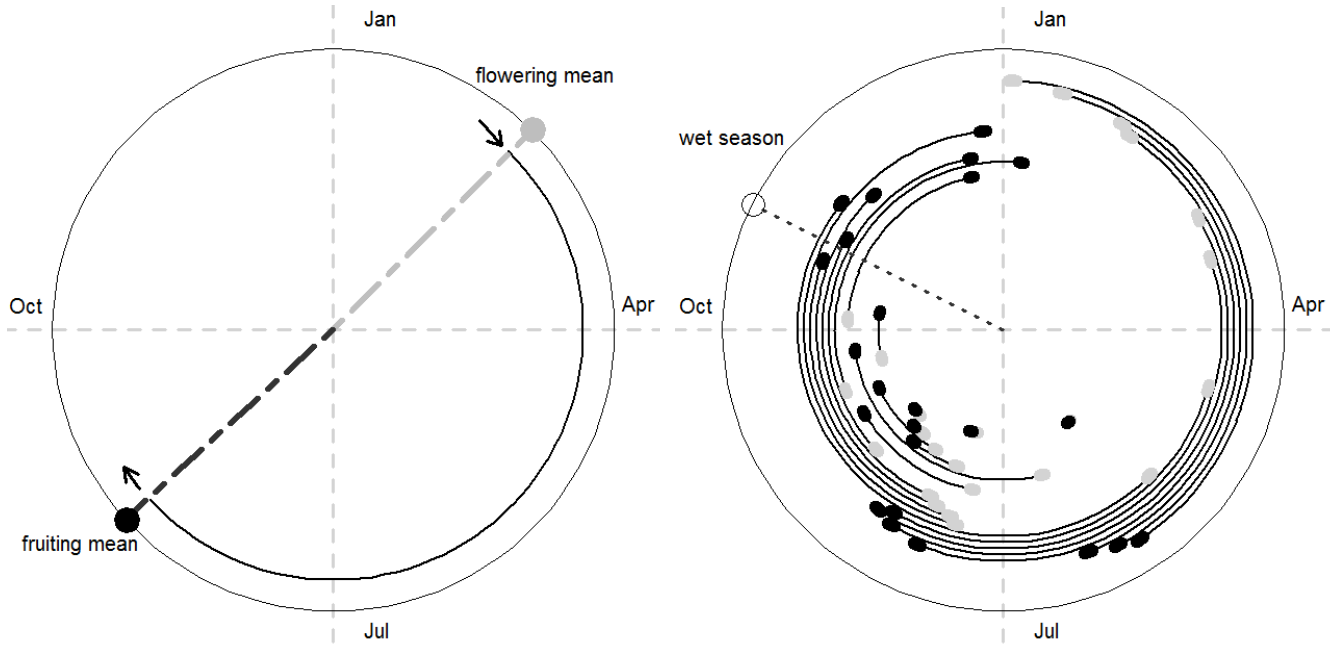


Figure 2. Maturation time; the length of maturation is estimated by circular distance (solid line) from the flowering mean (grey point) to the fruiting mean (black point). The right graph shows maturation lengths plotted across the year in relation to beginning of the wet season.

Geographical ancestral origins, climate ranges and flowering within genera

A total of 18 species were placed in the temperate ancestral origin group and 33 in the tropical group (Table 2.) The null hypothesis that flowering season and ancestral origin were independent could not be rejected. This indicated that many species from temperate origins now have a mean flowering day of year in autumn or winter months. Therefore, temperate ancestral origin was largely independent of flowering season (Fisher's Exact Test, p= 1.0).

Table 2. Origin and mean flower day of year; the circular mean flowering day of the 365 day year is recorded for each species. The mean in 360 circular degrees is recorded and an " * " is noted for species with a significantly non-uniform distribution of flowering day (Rayleigh test, $p < 0.05$). The origin assigned to each specie is also recorded

species	origin	mean flowering day	mean flowering angle (deg.)	mean fruiting day	mean fruiting angle (deg.)
Acacia koa	temperate	50	49*	206	203
Acacia koaia	temperate	34	33*	81	79
Antidesma platyphyllum	tropic	205	202*	301	296*
Antidesma pulvinatum	tropic	202	199*	317	312*
Argemone glauca	temperate	145	143*	145	143*
Bobea elatior	tropic	195	192*	355	350*
Bobea sandwicensis	tropic	211	208*	165	162
Broussaisia arguta	tropic	250	246*	2	1*
Cheirodendron platyphyllum	temperate	273	269*	329	324
Cheirodendron trigynum	temperate	203	200*	235	231
Coprosma foliosa	temperate	363	358	143	141*
Coprosma longifolia	temperate	72	71	216	213*
Coprosma ochracea	temperate	107	105*	212	209*
Diospyros sandwicensis	tropic	136	133*	309	304*
Elaeocarpus bifidus	tropic	20	19	6	5
Kadua acuminata	tropic	322	317*	31	30*
Kadua affinis	tropic	140	137	258	254
Kadua centranthoides	tropic	192	189*	240	236*
Melicope clusiifolia	tropic	61	60	211	208*
Melicope volcanica	tropic	200	197*	220	216*
Myrsine lanaiensis	tropic	338	333*	135	133
Myrsine lessertiana	tropic	31	30	160	157*
Myrsine sandwicensis	tropic	15	15*	153	150*
Nestegis sandwicensis	temperate	290	286*	112	110*
Perrottetia sandwicensis	tropic	10	9*	184	181
Pipturus albidus	tropic	240	236*	265	261
Pisonia sandwicensis	tropic	258	254*	279	275*
Pittosporum confertiflorum	tropic	44	43*	177	174
Pittosporum glabrum	tropic	49	48*	228	224
Polyscias hawaiensis	temperate	231	228*	179	176
Polyscias kavaiensis	temperate	231	227*	238	234
Polyscias oahuensis	temperate	197	193*	292	288*
Psychotria kaduana	tropic	170	167*	289	285
Psychotria mariniana	tropic	166	164	263	259*
Santalum ellipticum	temperate	183	180*	359	354
Santalum freycinetianum	temperate	186	183*	228	224
Sapindus oahuensis	tropic	214	210*	55	54
Scaevola gaudichaudiana	tropic	270	266	270	266
Scaevola gaudichaudii	tropic	219	215	219	216*
Scaevola mollis	tropic	220	217*	220	216
Smilax melastomifolia	tropic	75	73*	217	214
Sophora chrysophylla	temperate	34	33*	203	200*
Syzygium sandwicense	tropic	275	271*	349	344*
Touchardia latifolia	tropic	196	193*	194	191*
Vaccinium calycinum	temperate	46	45*	171	168*
Vaccinium dentatum	temperate	216	212	218	215*
Vaccinium reticulatum	temperate	226	223	210	207*
Wikstroemia oahuensis	tropic	211	208	246	242*
Wikstroemia sandwicensis	tropic	205	202*	215	212
Wikstroemia uva-ursi	tropic	192	188	1	0
Xylosma hawaiiense	tropic	228	225*	350	345*

Species in consistently wet environments were not more likely to have a unimodal distribution of flowering day than species in dry seasonal environments. The null hypothesis that seasonally dry environments are independent of seasonal flowering could not be rejected (Fisher's Exact Test, $p = 0.1932$). While not statistically significant, slightly more species in wet environments were found to have uniform distributions with flowering throughout the year.

Flowering means were not entirely constrained within genera (Fig. 3) (Watson-Wheeler Test, $p = 0.2304$). Species pairs of the same genus with non-uniform distributions had a sample size of $n = 11$ and species from different genera and with non-uniform distributions had a sample size of $n = 25$. While most congeneric pairs had a very similar mean flowering day of year, sometimes a third species in the genus had a very different mean flowering day (Fig. 3).

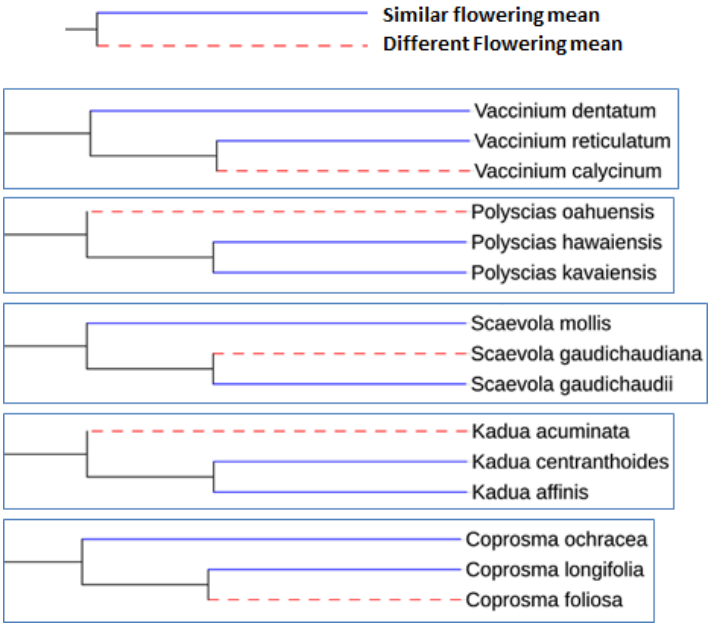


Figure 3. Phylogenetic trees of selected genera; two species within a genus show a very similar mean flowering day of year (solid line), while other species in the same genus have a very different flowering mean (dashed line). Phylogenetic tree constructed with PhyloT (National Center for Biotechnology) (phylot.biobyte.de/contact.html) and visualized using FigTree (tree.bio.ed.ac.uk/software/figtree).

Discussion

Most of the species in this study showed peak flowering in July (Fig. 1). Flowering in mid-summer months has been associated with the solar maxima in other temperate and tropical studies (van Schaik et al. 1993; Zimmerman et al. 2007). Flowering at about the time of the solar maxima allows plants to allocate assimilates directly to flowers rather than expending extra energy to store assimilates for later flower production (Chapin et al. 1990). Species such as *Acacia koa* and *Sophora chrysophylla* that had peak flowering in January and February may flower earlier in the year in order to synchronize their fruiting to the beginning of the wet season. These species may also be more efficient with slightly lower levels solar radiation. McDaniel & Ostertag (2010) found higher levels of light did not have a significant effect on the growth of *Acacia koa* and *Sophora chrysophylla* while other Hawaiian species did show more growth with higher light levels. These two species also have symbiotic relationships with nitrogen-fixing bacteria that may facilitate winter flower production (Lawrie 1974; Nakao et al. 1996).

Most species in this study had a fruiting mean during August at about the onset of the rainy season in the Hawaiian Islands (Fig. 1; Fig 2). This pattern of fruiting near the beginning of the wet season is consistent with other tropical forests (van Schaik et al. 1993; Sakai 2001). I also found that species with longer maturation times flower earlier in the year, which indicates support for the optimal time of germination hypothesis (van Schaik et al. 1993). Fruiting near the onset of the wet season may be an adaptation to dispersal and recruitment selection pressure (van Schaik et al. 1993). Interestingly, the Hawaiian wet season, *Ho'oilo*, is translated as, the cause of (*Ho'o*), germination (*ilo*) (Ziegler 2002).

Plants occurring in more seasonally dry environments did not show a statistically significant trend in having more seasonal flowering than species in consistently wet

environments. Seasonal flowering is common in tropical dry forests however (van Schaik et al. 1993). About 85% of plants in locations with a dry season did show seasonal flowering whereas about 60% in consistently wet environments showed seasonal flowering. Several common Hawaiian dry forest species were not included in this study however. A larger sample size that included an equal number of species from better defined dry forest regions may show more frequent seasonal patterns in dry forest plants.

Forrest & Miller-Rushing (2010) discuss possible mechanisms for evolutionary changes in phenology over time and discuss how phenotypic plasticity, selection pressures and genetic constraints complicate interpretations of evolutionary mechanisms in addition to genetic drift and mutation. Many of the original colonists of the Hawaiian flora radiated into several species and became morphologically diverse while showing relatively low genetic differentiation (Keeley & Funk 2011). Other plants of the Hawaiian flora have not speciated since colonization (Keeley & Funk 2011). Since 7 of the 18 species (37%) from temperate ancestral origins flowered during fall or winter months in this present study, there was no statistical evidence that suggested temperate species retained their spring and summer seasonal flowering. In some cases, these species may have retained traits associated with flowering in response to temperature cues, in which case, winter temperatures in the Hawaiian Islands could more closely match that of summer temperatures in more northern latitudes. Many northern latitude regions have summer temperatures that even exceed the summer temperatures in the Hawaiian Islands however.

Two of the temperate species were in the genus *Vaccinium*. Species in the genus *Vaccinium* are currently thought to have colonized the Hawaiian Islands from boreal regions (Kron et al. 2002). If these shrub species retained an ancestral trait for vernalization, the lack of cold temperatures in the autumn months may have facilitated continual flowering in the

Hawaiian Islands (Delpierre et al. 2016) and two of the three *Vaccinium* species showed uniform flowering throughout the year. Future investigation analyzing correlations between flowering and cold temperatures may disentangle the effect of these phenology cues for *Vaccinium* in the Hawaiian Islands. Of the three *Vaccinium* species in this study, *V. calycinum* and *V. reticulatum* are phylogenetically more closely related to each other than to *V. dentatum* (Fig. 3). The species *V. dentatum* and *V. reticulatum* appear to flower throughout the year and do not have a distinct peak but their mean flowering days were strikingly similar in July at day 212 and 222 respectively. Interestingly, the more recently derived *V. calycinum* has significantly unimodal pattern and has a mean flowering day in a February rather than during the summer. Like many plants in the Hawaiian flora, these *Vaccinium* are fleshy fruited species and are bird dispersed.

One possible cause of such different flowering times could be selection by native Hawaiian birds such as the Hawaiian honeycreepers. Fleshy fruits may be consumed and seeds dispersed to locations that isolated breeding between populations. This mechanism has been proposed for the speciation of Hawaiian taxa in the genus *Cyanea* (Givnish et al. 2009). Another cause of flowering time divergence might be selection to avoid pollinator competition. The dominant Hawaiian canopy tree species ʻōhiʻa lehua, (*Metrosideros polymorpha*) and the dry forest canopy species māmane (*Sophora chrysophylla*) are suggested to have staggered flowering times in order to reduce pollination competition (Carpenter 1976). Many congeneric species in the present study flowered at a remarkably similar time of year, which may suggest that the benefits to sharing pollinators are greater than competition for pollinators. Breeding isolation due to a pollination mechanism cannot be rejected for some congeneric species that flower at very different times of year however.

Several congeneric species in this study showed a remarkably similar mean flowering day

of year (*Antidesma, Bobea, Pittosporum, Polyscias, Psychotria, Santalum, Scaevola, Vaccinium, Wikstroemia*) (Table 2). All of the endemic Hawaiian species pairs in this study had speciated from a single colonizing ancestor with the exception of one pair. The two species from the genus *Santalum* colonized the Hawaiian Islands by two separate colonization events (Harbaugh & Baldwin 2007). Although ancestors of the two species in *Santalum* colonized the Hawaiian Islands from different ancestors at different times (1 and .5 million million years ago), they share a mean flowering day of year within 3 days of each other. This degree of similarity appears to be consistent with phylogenetic conservatism of flowering phenology.

Congeneric species in this study were also mostly sympatric (Price et al. 2012), or are at least β sympatric across large habitats, although microhabitats or niche spaces are not entirely known (Ackerly et al. 2006). Therefore, the mechanism for different flowering times within genera is unclear. If competition was strong between closely related species as Darwin (1859) suggested, then flowering times would be expected to differ among sympatric congeneric species. If there was an ecological advantage to having similar flowering times, such as shared pollinators (Moeller 2004), or if flowering was strongly phylogenetically conserved (Du et al. 2015), then flowering times would be mostly similar throughout sympatric congeners because they radiated from the same colonizations. Cantley et al. (2016) also found both similar and different flowering times within the genus *Coprosma* in the Hawaiian Islands. The species *C. ochracea* and *C. rhynchocarpa* flowered in spring on Hawaii Island while *C. cordicarpa* and *C. stephanocarpa* flowered in summer and winter months respectively. A study by Papadopulos et al. (2011) suggested differing flowering times had driven speciation in *Coprosma* and *Metrosideros* on Lord Howe Island in the Pacific. The differences in flowering times were thought to be associated an elevation gradient because species at lower elevations tended to

flower earlier, and this might have isolated breeding (Papadopulos et al. 2011). Long-term reciprocal transplant experiments or greenhouse experiments could be conducted to confirm whether these retained a possible phenotypic trait and would flower at the same time of year when placed in the same temperatures. Further investigations are needed to understand the specific mechanisms driving inconsistencies in flowering patterns of congeneric species. These mechanisms would reveal important evolutionary processes that have structured the phenology in Hawaiian plants.

Conclusions

This study demonstrated that herbarium specimens, along with circular statistics, can be used to analyze phenological patterns of tropical flora. Plants in the Hawaiian Islands appear to flower at a time of year that synchronises their fruiting with the onset of the wet season. Several lineages that colonized the Hawaiian Islands from temperate regions appear to have evolved to flower outside of spring and summer seasons. While some species in the same genera show a remarkably similar mean flowering day of year, other congeners show a very different mean flowering day. Further investigations into the phenology of Hawaiian plants are needed to better understand the evolutionary processes that shaped their current flowering patterns.

CHAPTER 3

PHENOLOGICAL RESPONSES TO CLIMATE CHANGE IN THE HAWAIIAN FLORA

Abstract

Herbarium records are widely relied upon to investigate correlations between long-term rising temperatures and flowering phenology. Temperature and rainfall are known to affect the flowering phenology of tropical plants but responses to long-term changes in rainfall are under studied. I investigated associations between flowering and rainfall and associations between flowering and temperature for t Hawaiian taxa. I investigated phenological patterns of 51 endemic Hawaiian angiosperms using herbarium records, geographical information system data, and circular statistics. A total of 5,517 herbarium records collected from 1895-2015 were analyzed. Circular-linear regression estimates for changes in flowering day were about 5.0 days $°C^{-1}$ and about 0.3 days mm^{-1} rainfall. Analysis of long-term changes showed flowering day was earlier over time for 22% of these species and was delayed for 12%. Long-term changes in flowering were estimated to be about 0.64 days $year^{-1}$. This study demonstrated that herbarium records, geospatial grid data, and circular statistics are valuable resources for understanding how long-term changes in temperature and rainfall are associated with flowering phenology in tropical plants.

Introduction

Herbarium records have been used to investigate the effects of climate change since Primack et al. (2004) conducted a study analyzing phenological changes in the temperate region of the North-Eastern United States. Specifically, herbarium specimens are often used to investigate how the day of year that flowering occurs changes over long periods of time (Primack et al. 2004; Primack & MIller-Rushing 2012; Davis et al. 2015). Studies have also used herbarium specimens to show correlations between temperature and the day that flowering occurs (Gallagher et al. 2009; Panchen et al. 2012). Studies that use herbarium specimens to investigate phenological associations with climate change are primarily conducted in temperate regions where it is easier to maintain a cool dry environment for herbarium specimen preservation (Primack & MIller-Rushing 2012; Davis et al. 2015). To date, no study has used herbarium specimens to investigate long-term phenological changes in a tropical region. Most studies suggest phenology in tropical plants is primarily influenced by precipitation or photoperiod (Cleland et al. 2007; Borchert et al. 2005). While global climate change is well documented, climate change in tropical regions is less pronounced (Cleland et al. 2007). Climatological studies in the Hawaiian Islands however, have found evidence for both rising temperatures and reduced rainfall over time, especially at high elevations (Giambelluca et al. 2008; Longman et al. 2015).

Early studies investigating flowering phenology in the Hawaiian Islands have been limited to field surveys over the course of approximately two years (Lamouroux 1970; van Riper 1980; Berlin 2005). I addressed the lack knowledge for long-term phenological patterns in the Hawaiian Islands by utilizing long-term herbarium specimen records. I investigated correlations between long-term temperature and precipitation data with and flowering dates from specimens.

I hypothesized that the day of year that flowering occurs would be earlier over time because this trend is globally dominant in flowering plants (Parmesan & Yohe 2003; Cleland et al. 2007). I also hypothesized that estimates of both rainfall and temperature from herbarium specimen collection sites would be correlated with the flowering day of year. Rainfall is predominantly associated with flowering in tropical regions and rainfall is decreasing in the Hawaiian Islands (Brearley et al. 2007; Longman et al. 2015). It was expected that responses to climate from regression estimates would be consistent with long-term changes in flowering.

Methods

Herbarium data

A total of 14,050 herbarium specimens were examined for the presence of flowers at The Bernice Pauahi Bishop Museum, Hawaii (BISH) from October 2015 to January 2016. Only specimens with flowers, locations and dates were used for analyses. A final count of 5,517 records (1895-2015) were used in analyses for 51 endemic Hawaiian species. Duplicate specimens were removed from the analysis (Rumpff et al 2010). Global Positioning System (GPS) points were manually assigned to each specimen record using the intersection of the trail and the elevation from the specimen label information (Edwards and Still 2008). Coordinates were assigned using topological map layers in ArcGIS (10.3.1) (USA Topo Maps, National Geographic Society 2013) (see also CHAPTER 2, Methods).

Temperature data

Temperature data were collected from the Climate Atlas of Hawaii (Giambelluca et al., 2013). Gridded raster layers (250 m x 250 m resolution) of mean monthly air temperatures were

downloaded in ERSI format. Monthly averages of temperature data were collected from the National Oceanic and Atmospheric Association (NOAA) NOWData system for the "Honolulu Area" from 1895-2015 in table data format. The long-term NOAA records were merged with the gridded raster layers to create new ERSI grid layers for 1895-2015. For example, when Honolulu Area temperature averages were low in January for the year 1900, then it was expected that temperature averages were also likely to be low for the Konahuanui summit in January of 1900. Therefore the long-term differences in January temperature averages between the Honolulu Area and the Konahuanui summit were used to estimate the temperature averages for the Konahuanui summit in January of 1900. Temperature values were extracted at each assigned GPS point from the new 1895-2015 grid layers using ArcGIS (v. 10.3.1) (Gallagher 2009).

Rainfall data

Rainfall data were also collected from the Climate Atlas of Hawaii (Giambelluca et al., 2013). Gridded raster layers (250 m x 250 m resolution) for long-term mean annual rainfall and long-term mean monthly rainfall were used in analyses. Gridded raster layers (250 m x 250 m resolution) for monthly rainfall totals from 1920-2012 were available (Frazier et al. 2016) so no new layers needed to be created. Rainfall values were extracted at each assigned GPS point of the 1920-2012 grid layers using ArcGIS (v. 10.3.1).

Analyses

Circular statistical methods were used to analyze the distribution of flowering dates and the mean flowering day of year for each species (Circular package, R programing language). The circular

mean flowering day of year was determined across all species using only species with significantly non-uniform distributions of flowering day of year (Rayleigh test $p < 0.05$).

Circular-linear regressions were conducted to analyze the relationships between temperature (independent) and flowering day of year (dependent). A circular-linear regression was also conducted to analyze the relationships between rainfall (independent) and flowering day of year (dependent). Since temperature and rainfall are often correlated, multiple regression analyses were not performed with both temperature and rainfall as predictors in order to avoid problems of multicollinearity.

Many studies analyzing species in temperate regions have used temperature averages from 3 months prior to the date of flowering for all species. I analyzed 5 different climate averages prior to flowering and found the climate average with the strongest correlation for each individual species instead of using averages from 3 months prior for all species. Temperature averages and rainfall totals were collected from 1, 3, 6, 9 and 12 months prior to the species mean flowering day of year (Rawal et al. 2015). For each individual species, I found the circular-linear correlation between flowering day of year and all 5 climate average periods. The strongest correlation coefficient of the 1, 3, 6, 9, and 12 month averages was then used in further analyses.

In order to analyze changes in flowering day of year over time, circular-linear regression analyses were conducted between the specimen collection year (independent variable) and the flowering day of year (dependent variable). Some species showed drastic responses to climate variables on the circular scale. Therefore, to further examine species with drastic estimates, I also conducted a linear regression between the collection year (independent variable) and the

distance in days (dependent variable) that the collection date was from the mean flowering day for each species (Primack, et al. 2004).

I also analyzed the effect of temperature and rainfall on the mean flowering day of year by discretizing each climate variable into two groups; 1) a high temperature or rainfall group and 2) a low temperature or rainfall group. Groups were chosen by dividing the temperature or rainfall samples at the median of the sample in order to maximize the equality of the discretized groups. A significantly non-uniform mean flowering day of year was determined for each high and low group (Rayleigh Test ($p<.05$)). High and Low groups that did not meet a significantly non-uniform test were not used in the analysis. For example, the mean flowering day of year value for the high temperature group was compared to the mean flowering day of year value for the low temperature group for each species, but only if both groups had a significantly non-uniform flowering distribution (Watson-Williams t-Test ($p<.05$)).

Changes in flowering day over time were also analyzed for each species by comparing means of two groups; 1) a "historic" group and 2) a "current" group (Primack 2004; Bertin 2015; Searcy 2012). The historic group consisted of collection dates that were more distant in the past and the current group consisted of dates that were more recent. Searcy et al (2012) defined a historic group between 1860-1952 and a current group between 1999–2007, whereas Bertin (2005) divided records at 1980. Primack et al. (2004) used a historic group from 1900-1920 and defined a current group with field data from 1980-2002. I choose historic and current groups by dividing records at the median collection year for each species. This method maximized the equality of the sample lengths of the discretized groups. The median collection year for each species ranged from about 1930 to about 1980. Dividing records at the median year captured historic and recent climate changes over the last century in Hawaii (Giambelluca et al. 2008,

Longman et al. 2015). A significantly non-uniform mean flowering day of year was determined for each group (Rayleigh Test ($p<.05$)). Historic and current groups that did not meet a significantly non-uniform test were removed from analyses. I compared the mean flowering day of year between the historic and current group for each species to determine long-term changes over time (Watson-Williams t-Test ($p<.05$)).

Results

Temperature

A total of 45 species (90%) showed a significant correlation with one or more temperature period averages (Fig. 4; Table 3). There was a significant negative correlation for 21 species and significant positive correlation for 24 species (Fig 4.). The average negative response to temperature was -5.24 days $°C^{-1}$ (range [-6.81,-3.93] days $°C^{-1}$), while the average positive response to temperature was 5.15 days $°C^{-1}$ (range [3.10, 7.84] days $°C^{-1}$). The overall average response to temperature in days was about 5.20 days $°C^{-1}$. Most species showed the strongest correlations with temperature averages 3 prior to flowering.

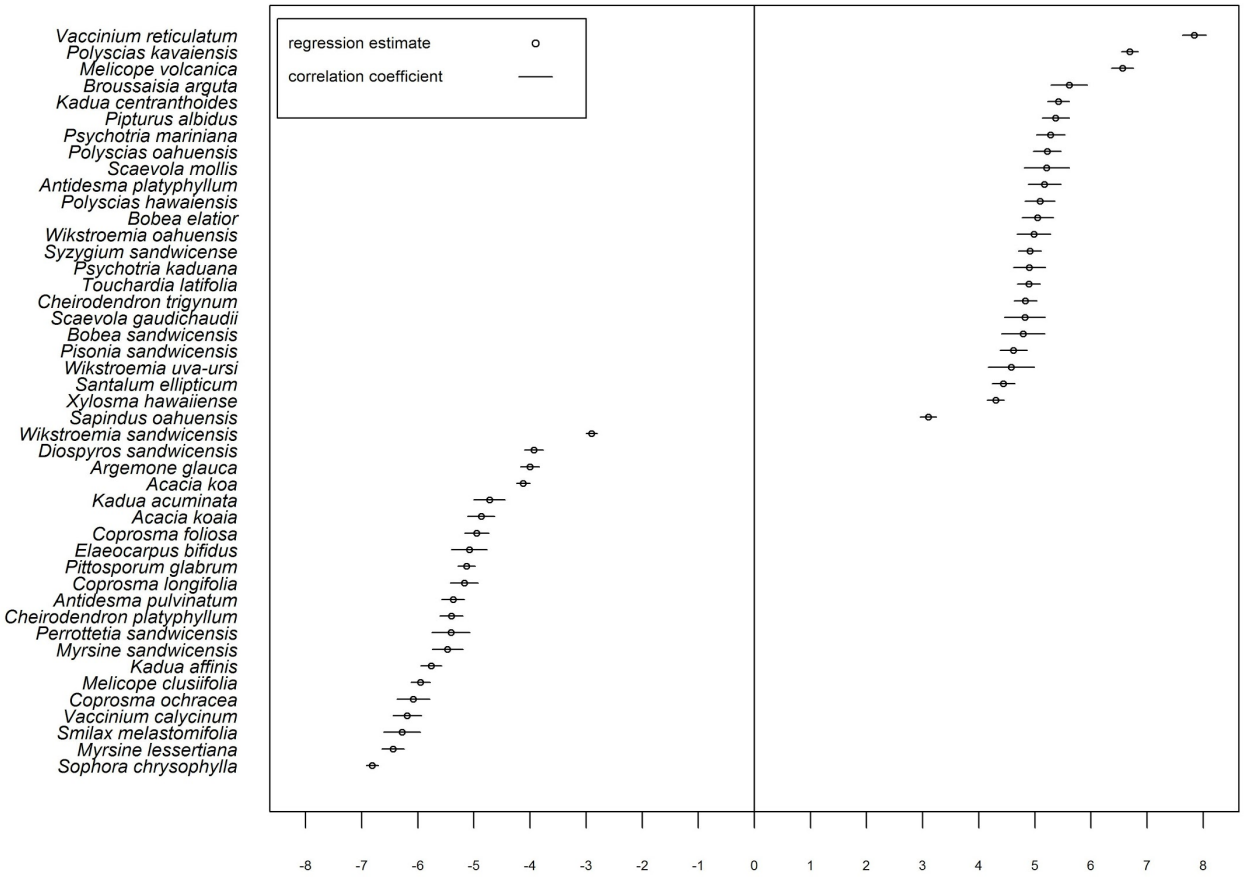


Figure 4. Temperature regression estimates (days $°C^{-1}$) and correlation coefficients (represented by bar width). Flowering day shifted -5.24 and 5.15 days $°C^{-1}$ on average. The average of the correlation coefficients were -0.26 and 0.34.

Rainfall

A total of 27 species (53%) showed significant correlation ($p < 0.05$) with 1 or more rainfall totals (Fig. 5; Table 3). Flowering day delay with lower rainfall was significant for 17 species and flowering day advancement with lower rainfall was significant for 10 species (Fig. 5; Table 3). The average positive response to rainfall was 0.34 days mm^{-1} (range [0.05,0.82] days mm^{-1}), while the average negative response to temperature was -0.26 days mm^{-1} (range [-1.92, -0.03] days mm^{-1}). The overall average response to rainfall in days was about 0.30 days mm^{-1}. Absolute values of correlation coefficients were on average lower for rainfall than for

temperature at 0.16 and 0.35 respectively. Most species showed strongest correlations with rainfall totals 12 month prior to flowering.

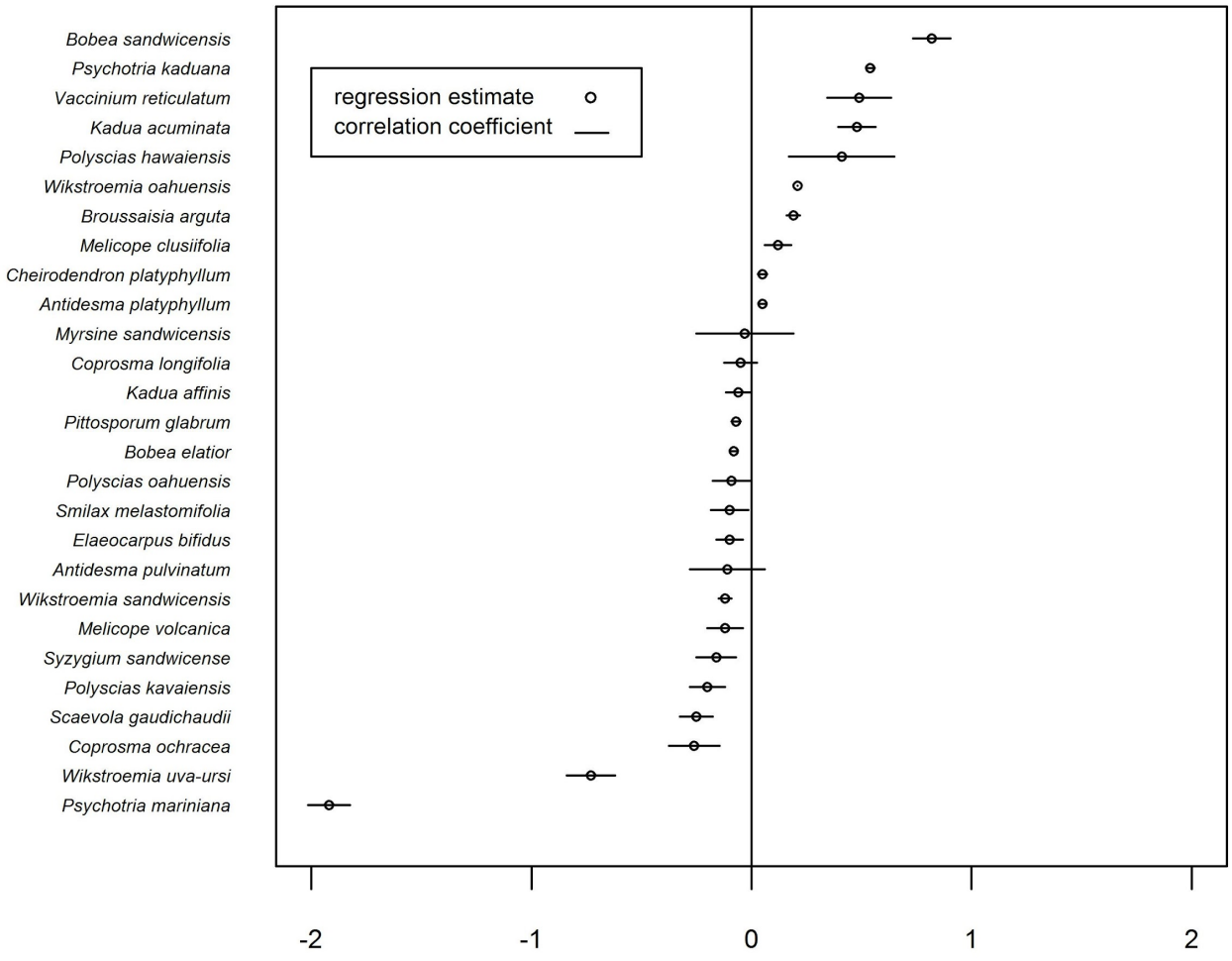


Figure 5. Rainfall regression estimates (days mm^{-1}) and correlation coefficients (represented by bar width). Flowering day shifted -0.33 and 0.38 days mm^{-1} on average. The average of the correlation coefficients were -0.17 and 0.14.

Long-term change

Of the 51 species in this study, 17 showed changes in flowering day of year over time. A total of 11 species (22%) showed earlier flowering over time and 6 species (12%) showed later flowering over time (Fig. 6; Table 3). The average regression estimates were -0.66 days yr^{-1} for negative responses and 0.62 days yr^{-1} for positive responses. The overall average regression response was

0.64 days yr^{-1}. Using these regression estimates, overall long-term rate for change in flowering day of year was between about 2 and 100 days over the past 100 years (Fig. 7). Four species with low sample sizes had very high and imprecise regression estimates (above 2.8 days yr^{-1}), and they were not considered further. For these species, the model estimated flowering changes across a response variable length that was beyond one year (360°). Three of the four species that were removed for inflated circular-linear regression estimates showed a significant change in flowering over time when analyzed by the method using distance in days from the mean flowering day. The distance from mean method used distance in days (dependent variable) between the collection date of the specimen and the mean flowering day of each species. This distance in days was then regressed on years (independent variable) (Primack et al. 2004). Using the distance from mean model, these four species showed an average advancement of 0.64 days yr^{-1} (Table 3).

Several species also showed long-term phenological changes when discretized groups were analyzed. Differences in mean flowering between historic and current groups were found for 14 species (27%). Of the 14 species showing differences between historic and current groups, 6 were found to have an earlier flowering mean in the current group and 8 were found to have a later flowering mean in the current group. These results indicate that some species flowered later in the year over time and some flowered earlier in the year over time.

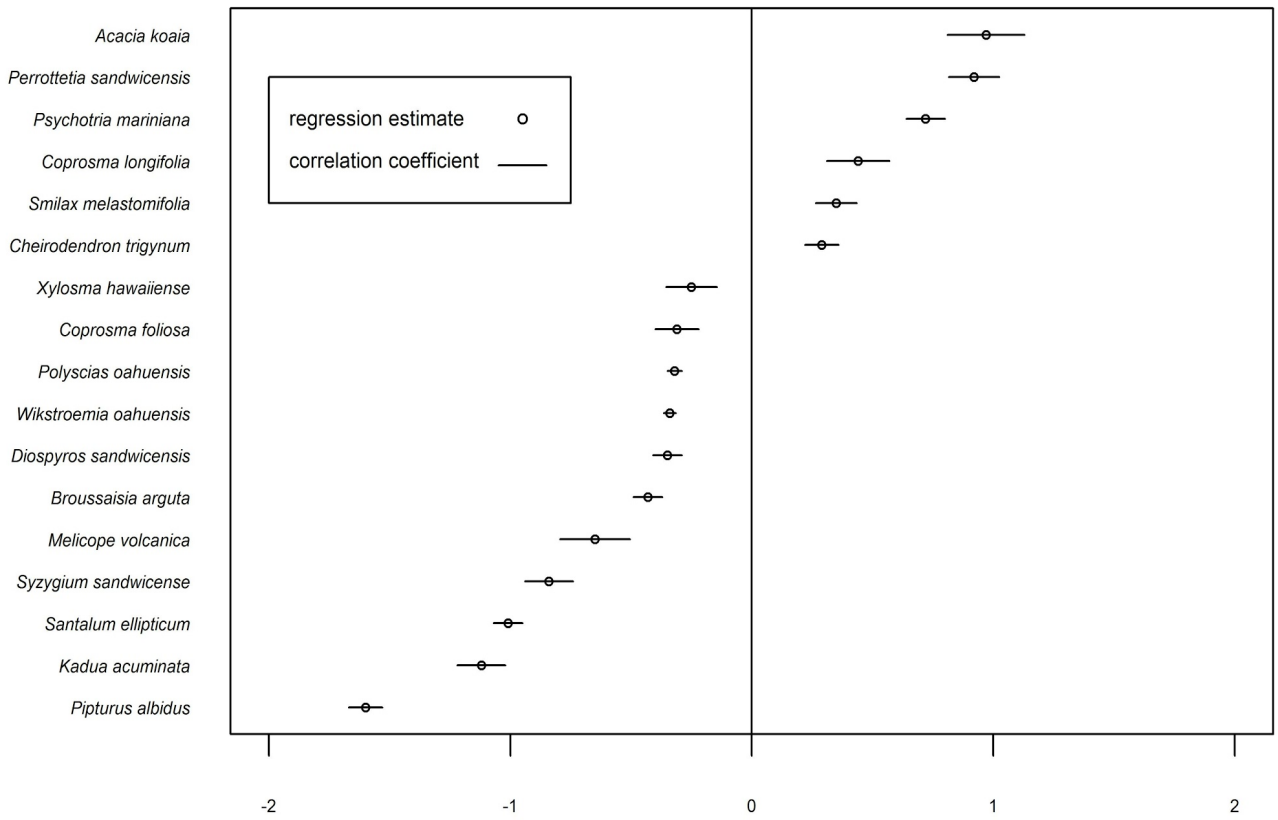


Figure 6. Long-term regression estimates (days yr^{-1}) and correlation coefficients (represented by bar width). Flowering day shifted -0.66 and 0.62 days yr^{-1} on average. The average of the correlation coefficients were -0.15 and 0.21.

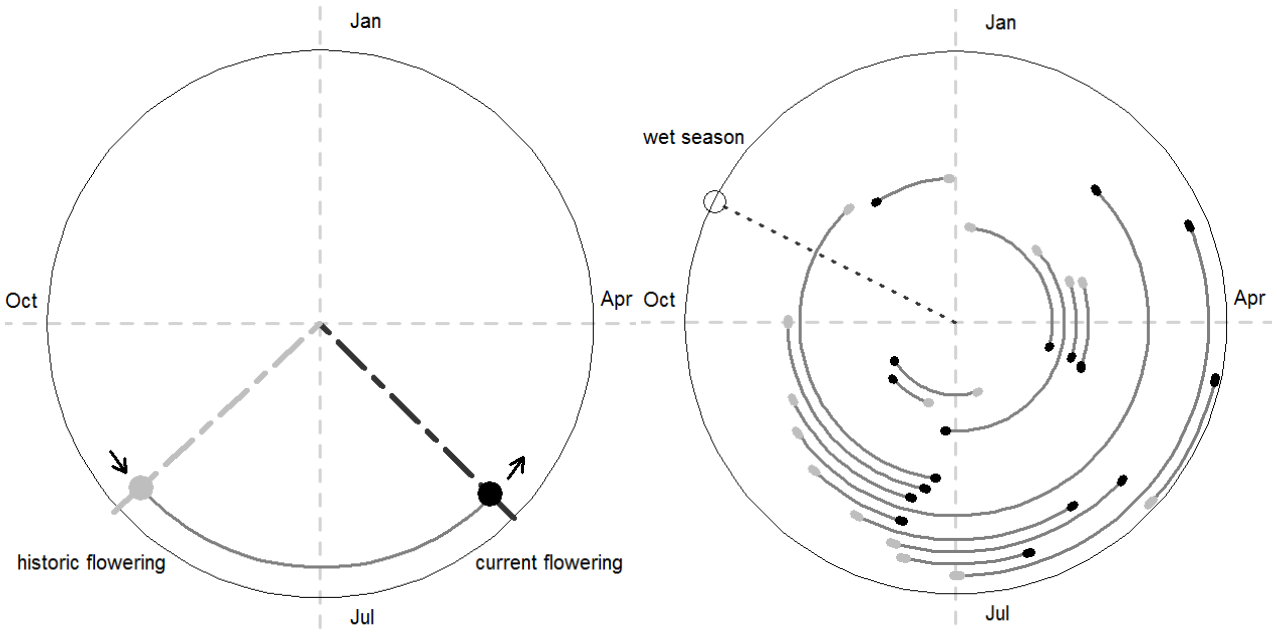


Figure 7. Long-term changes in days are plotted using estimates from circular-linear regressions over 100 years for each species. The historic flowering time points are plotted in grey and the current flowing time points are plotted in black.

Consistency and Inconsistency

Two main approaches were used to test for links between phenology and climate, 1) GPS points were used to identify climate conditions at each collection locality and these climate data were tested for a relationship to flowering, and 2) flowering date among historic collections was compared to flowering date among recent collections to test for changes over time. Ideally, both approaches should detect the same relationship between phenology and climate (temperature or rainfall). For example, if a species shows a negative correlation between flowering time and temperature, based on temperatures at the GPS collection points, it should also show earlier flowering over time when temperatures are rising over time among recent collections, in comparison to historic collections.

A total of six species that showed responses to temperature were consistent with long-term phenology changes. Four flowered earlier over time and two flowered later over time

(Table 3). A total of seven species that showed responses to rainfall were also consistent with long-term phenology changes from circular-linear regressions. From discretized comparisons, four additional species were also consistent between responses to rainfall and long-term changes. Six of the species that showed consistencies between responses to rainfall and long-term changes flowered earlier over time and five flowered later over time. Only one species showed a long-term flowering change that was consistent with responses to both temperature and rainfall.

Eleven species were inconsistent between temperature and long-term changes. Three species were inconsistent between rainfall responses and long-term changes. Caution should be taken when interpreting these results. Defining flowering as earlier or later might be arbitrary on a circular scale for day of year. I assumed the that shifting flowering times would be represented by the shorter distance around the circle, and I chose the direction that would shift through the mean flowering day of year. Also, several of the discretized results were inconsistent with the flowering shift directions from circular-linear regression estimates for temperature responses. Rainfall responses, however, were completely consistent between discretized data and circular-linear regressions estimate directions. Regression estimates for changes over time were notably high, and had estimated a change in flowering day at about 64 days over 100 years (0.64 days yr^{-1}). A meta-analysis of 143 phenological studies by Root et al. (2003) estimated current rates of phenological change at about 5 days per decade, which would shift flowering about 50 days over 100 years. Since global climate change is accelerating, estimates across more recent time periods are higher. Rates of change for rainfall and temperature in the Hawaiian Islands over the last 40 years was higher than over the last 100 years (Giambelluca et al. 2008, Longman et al. 2015). A regression estimate of 0.64 days yr^{-1} would shift flowering about 26 days over 40 years.

Table 3. Consistencies and inconsistencies between regression estimates, comparisons of means and long-term changes.

Species	Temperature est. d/°C	Rainfall est. d/mm	Long-term circ/line est. d/y	Historic & Current group differences	Long-term lm est. d/y
Acacia koa	-4.12			33	
Acacia koaia	-4.87		0.97	82	
Antidesma platyphyllum	5.17	0.05			
Antidesma pulvinatum	-5.37	-0.11			
Argemone glauca	-4.00				
Bobea elatior	5.05	-0.08			
Bobea sandwicensis	4.79	0.82			
Broussaisia arguta	5.61	0.19	-0.43	56	
Cheirodendron platyphyllum	-5.40	0.05			
Cheirodendron trigynum	4.83		0.29		
Coprosma foliosa	-4.95		-0.31		
Coprosma longifolia	-5.17	-0.05	0.44		
Coprosma ochracea	-6.08	-0.26		94	
Diospyros sandwicensis	-3.93		-0.35		
Elaeocarpus bifidus	-5.08	-0.10			
Kadua acuminata	-4.72	0.48	-1.12	-130	
Kadua affinis	-5.76	-0.06		-47	
Kadua centranthoides	5.42				
Melicope clusiifolia	-5.95	0.12			
Melicope volcanica	6.56	-0.12	-0.65		
Myrsine lanaiensis					
Myrsine lessertiana	-6.44				
Myrsine sandwicensis	-5.47	-0.03			
Nestegis sandwicensis					
Perrottetia sandwicensis	-5.41		0.92	47	
Pipturus albidus	5.37		-1.60		-0.51
Pisonia sandwicensis	4.62				
Pittosporum confertiflorum					
Pittosporum glabrum	-5.13	-0.07			
Polyscias hawaiensis	5.09	0.41			
Polyscias kavaiensis	6.69	-0.20			
Polyscias oahuensis	5.22	-0.09	-0.32		
Psychotria kaduana	4.90	0.54		-100	-0.65
Psychotria mariniana	5.28	-1.92	0.72	49	
Santalum ellipticum	4.44		-1.01	-42	
Santalum freycinetianum					
Sapindus oahuensis	3.10				-0.50
Scaevola gaudichaudiana					
Scaevola gaudichaudii	4.82	-0.25			
Scaevola mollis	5.21				
Smilax melastomifolia	-6.28	-0.10	0.35	40	
Sophora chrysophylla	-6.81				-0.50
Syzygium sandwicense	4.91	-0.16	-0.84	-58	
Touchardia latifolia	4.89			-62	
Vaccinium calycinum	-6.19			119	
Vaccinium dentatum					
Vaccinium reticulatum	7.84	0.49			
Wikstroemia oahuensis	4.98	0.21	-0.34		
Wikstroemia sandwicensis	-2.90	-0.12			
Wikstroemia uva-ursi	4.58	-0.73			
Xylosma hawaiiense	4.30		-0.25		

Discussion

Changes in the flowering phenology of Hawaiian plants were associated with reduced rainfall over time. Rainfall has decreased in the Hawaiian Islands as much as 1 to 22 mm yr^{-1} from 1973-2012 (Longman et al. 2015). Therefore, low estimates suggest rainfall has declined about 40 mm in the last 40 years. Average estimates of flowering day response to rainfall in the present study indicate changes by about 0.30 days mm^{-1} of rainfall. Therefore, flowering day of year is estimated to have shifted between 12 and 260 days in the last 40 years due to changes in rainfall. Using estimated long-term changes in flowering at 0.64 days yr^{-1} indicates a flowering change of about 24 days over 40 years. The moderate consistencies between these models indicate that rainfall has a significant association with the phenology of many endemic Hawaiian plants. This trend is consistent with other studies that found tropical plant phenologies were influenced more by rainfall than temperature (van Schaik et al. 1993; Brearley et al. 2007; Cleland et al. 2007; Forrest 2010).

Recent high-end temperature change of about 0.27°C $decade^{-1}$ (Giambelluca et al. 2008) indicates a total change of about 1°C over the last 40 years in the Hawaiian Islands. Changes in flowering phenology are estimated to be about 5.20 days $°C^{-1}$ and indicate a total change of about 5.20 days over the last 40 years, or 1.30 days $decade^{-1}$ in response to temperature. This estimate for long-term phenological responses to temperature is slightly lower than global trends of about 2 to 5 days $decade^{-1}$ (Parmesan & Yohe 2003; Root et al 2003).

Estimates over a 40 year period indicate flowering day responses to rainfall were greater than temperature. Responses to rainfall were between 12 and 260 days while responses to temperature was about 5 days. A ten-year field study by Brearley et al. (2007) found rainfall was a more important influence for flowering than temperature for species in the Dipterocarpaceae

family in Central Borneo. Brearley et al. (2007) found different species in the same genus will flower in association with different climate cues, mainly in due to drought and the El Niño-Southern Oscillation (ENSO). The diversity of plant families in our present study may account for the variation in responses to temperature and rainfall across species.

A few early studies investigating phenology found similar phenological responses in Hawaiian plants. A two-year study by Lamouruex (1972) found *Acacia koa* flowering later at higher elevations with cooler air temperatures and earlier at lower elevations with warmer temperatures. Similarly, I found *Acacia koa* flowered earlier with warming temperatures. A study by van Riper III (1980) suggested *Sophora chrysophylla* flowered in February and the flowering mean for *Sophora chrysophylla* was also in February in the present study.

Several mechanisms may cause earlier or later flowering. Increases in photoperiod and temperature are known to advance flowering times. This has been shown experimentally for *Arabidopsis thaliana*, which regulates these cues with two different genes (Balasubramanian et al. 2006). Vernalization, a period of near freezing temperatures in winter months, is also known to advance flowering time for several temperate species (Martinez-Zapater & Somerville 1990; Kim et al. 2009; Darapuneni et al. 2014). Using herbarium specimens, Hart et al. (2014) found *Rhododendron* flowered later in the year when temperatures were warmer in fall months, likely due to the lack of a vernalization type mechanism. Drought has also been shown to be an important factor for the timing of flowering. Drought has delayed flowering in Mediterranean species (Llorens & Peñuelas 2017) and has also been the major cue for flowering in tropical species (Brearley et al. 2007). Further investigations will need to be conducted to determine specific mechanisms for earlier or later flowering in Hawaiian plants.

Several caveats should be considered when interpreting the findings of this study. The heterogeneity of topography and environmental conditions in the landscape to the Hawaiian Islands could facilitate flowering adaptations in subpopulations or varieties of these species. Since the ranges of these species span dry seasonal forests to wet montane rainforests, some individuals may respond differently to flowering cues due to genetic diversity (Berlin et al. 2000). For example, Berlin et al. (2000) studied two varieties of the dominant Hawaiian tree *Metrosideros polymorpha* and found they flowered at different times. Since many of the species in this present study are also common across the landscape, genetic variation may also play a similar role in the variation of flowering times. El Niño and La Niña patterns could also influence supra-annual flowering patterns which were not investigated in this present study. Although a previous study of Hawaiian plant phenology found only annual flowering patterns in Hawaiian plants (Berlin et al. 2000), it is likely that some Hawaiian plants have peak flowering patterns that span more than one year.

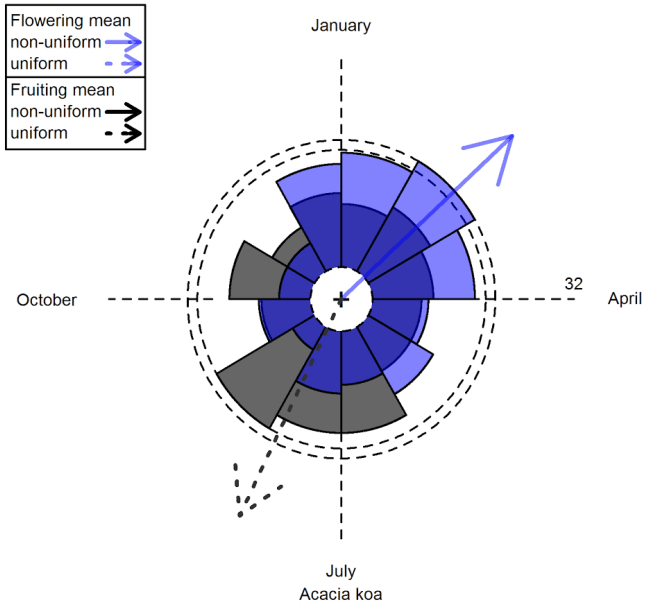
Climate driven changes in the phenology of endemic Hawaiian plants will likely affect several other species in the ecological network of the Hawaiian forest community. Impacts of climate change have been documented for Hawaiian avian species that are under the impending threat of extinction (Paxton et al. 2016). Changes in the flowering and fruiting phenology may increase stress on these populations. Krushelnycky et al. (2013) has also shown that after years of extensive management, the Hawaiian Silversword plant (*Argyroxyphium sandwicense*) remains threatened with extinction due to reduced rainfall over time. If contemporary selection induced by climate change has a greater effect on phenology that historical evolutionary processes, then recent climate change may significantly affect Hawaiian plants, even if some species do have flowering at low levels year-round.

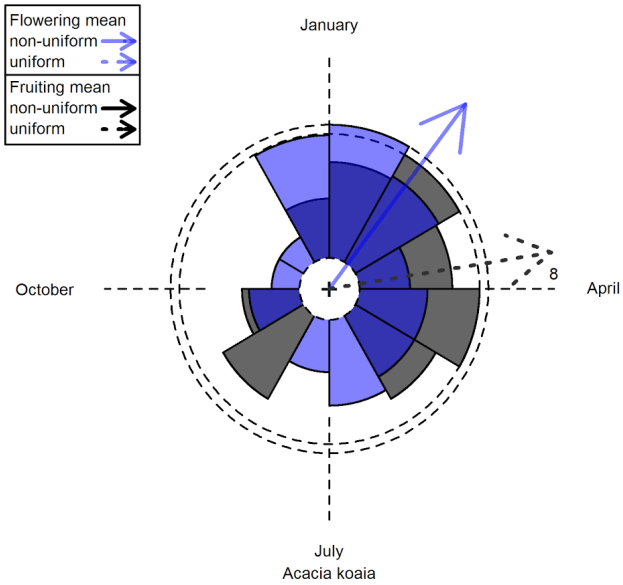
Conclusions

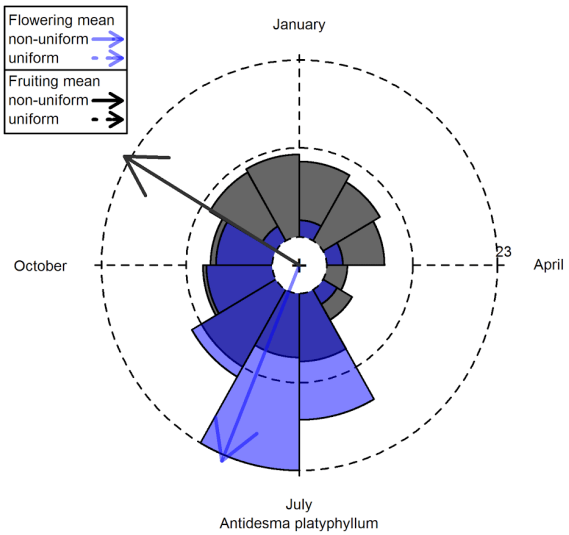
Long-term phenological responses to temperature and rainfall were found for several species of endemic Hawaiian plants. Based on recent climate change estimates for the Hawaiian Islands, flowering may have shifted as much as a month in response to rainfall and about 5 days in response to temperature over the last 40 years. This study demonstrated that herbarium records, geospatial grid data, and circular statistics are valuable resources for understanding how long-term temperature and rainfall changes are associated with phenological responses in tropical plants.

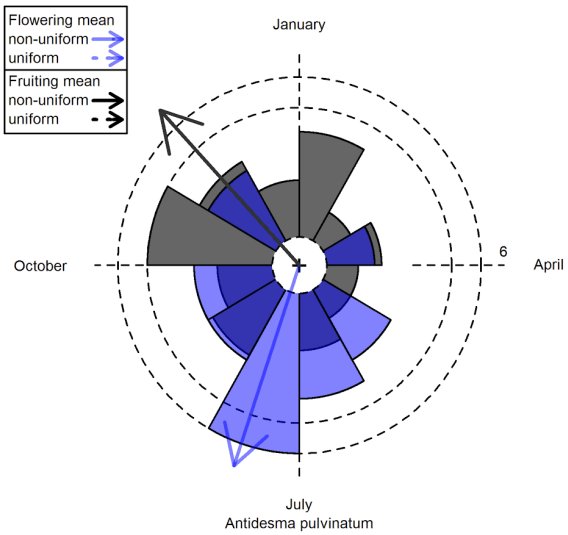
APPENDIX: FLOWERING AND FRUITING DAY MEANS AND DISTRIBUTIONS

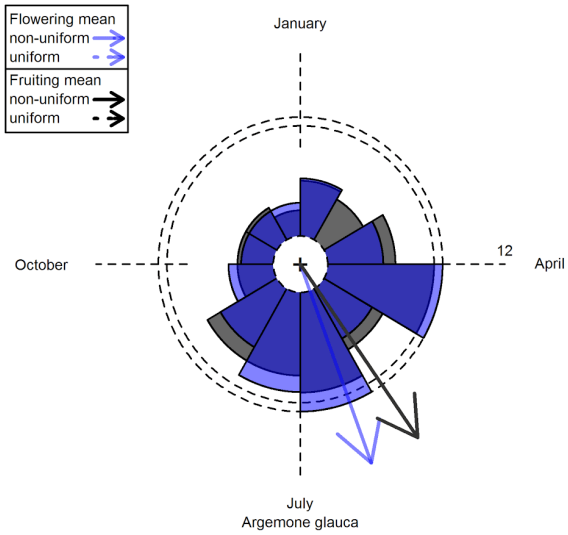
Flowering and fruiting day are plotted for each species using histogram windrose plots. Flowering is plotted in blue and fruiting is plotted in grey. The mean flowering day of year is represented by a blue arrow and the mean fruiting day of year is represented by a grey arrow. Each bin (petal) represents a month of the year. For each species, the highest frequency, or the radius of the outermost dashed circle, is recorded to the far right on the positive x-axis.



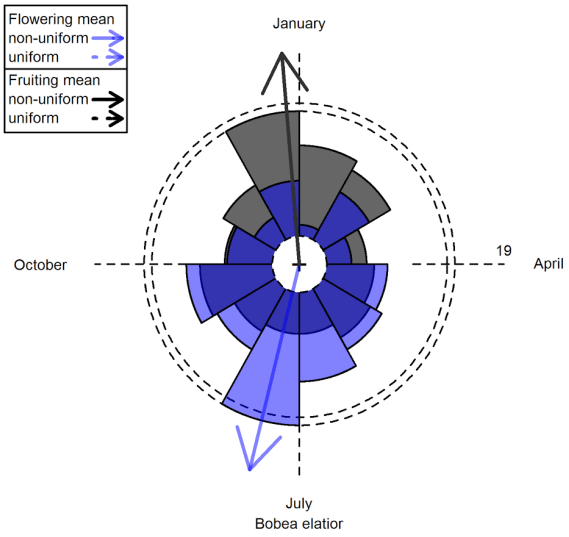




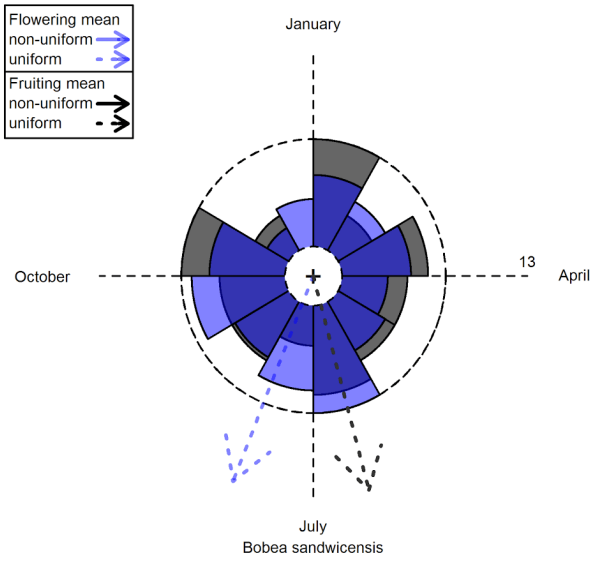




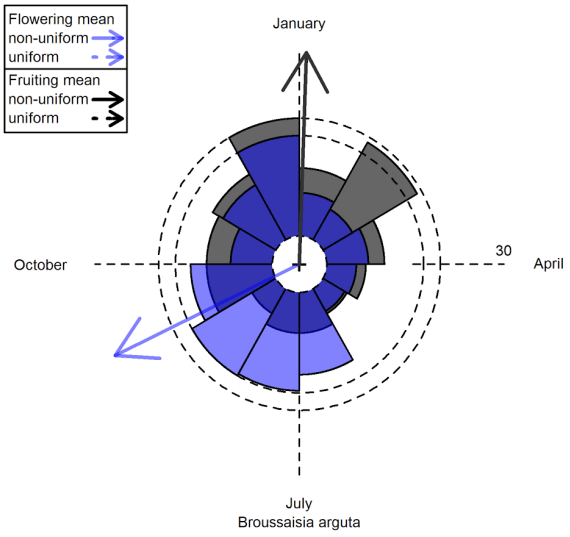
Argemone glauca



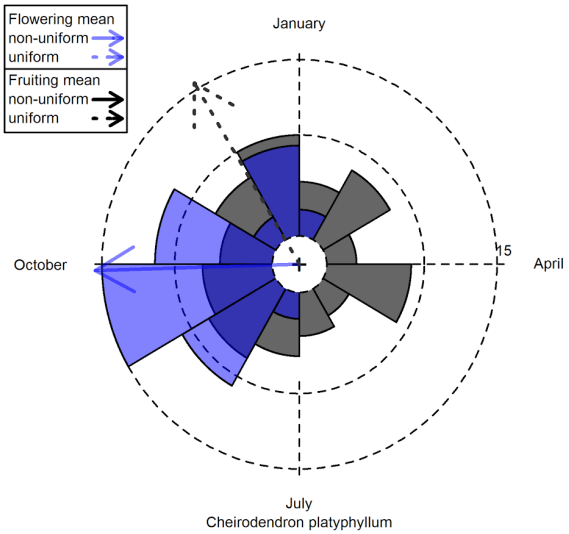
Bobea elatior



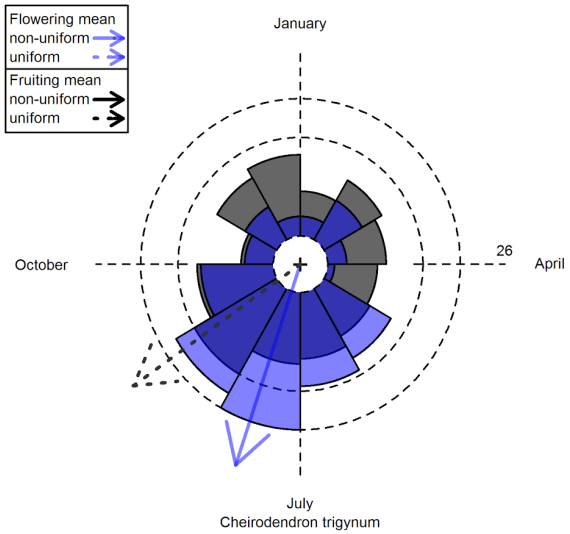
Bobea sandwicensis



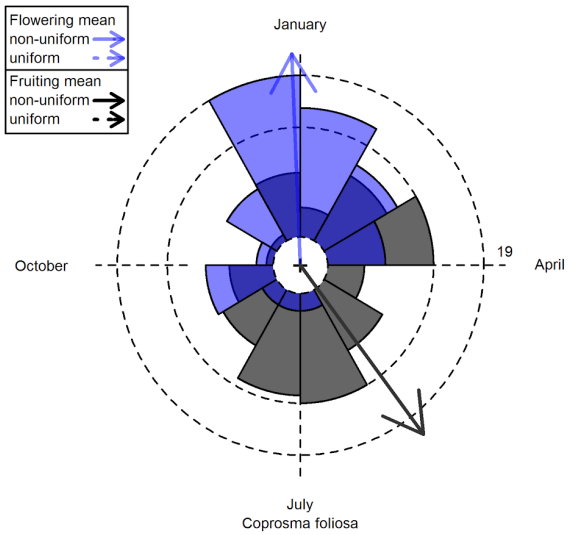
Broussaisia arguta



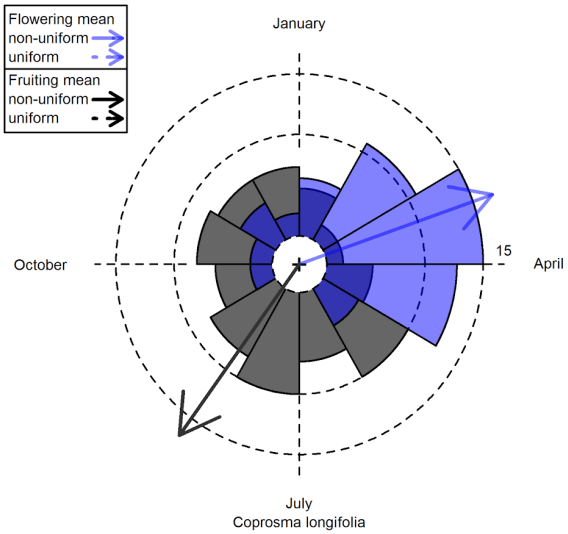
Cheirodendron platyphyllum



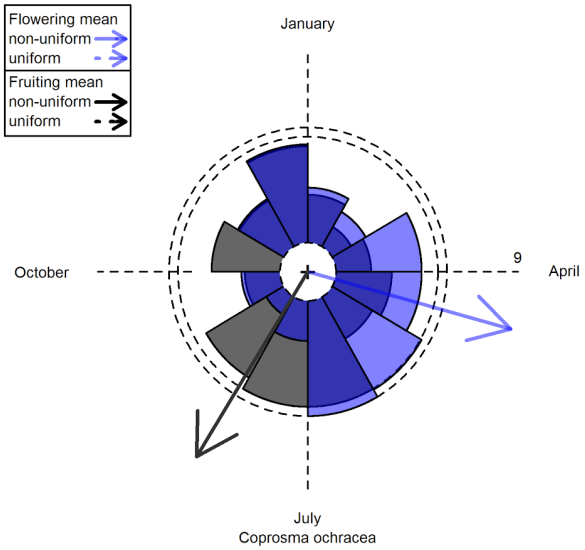
Cheirodendron trigynum



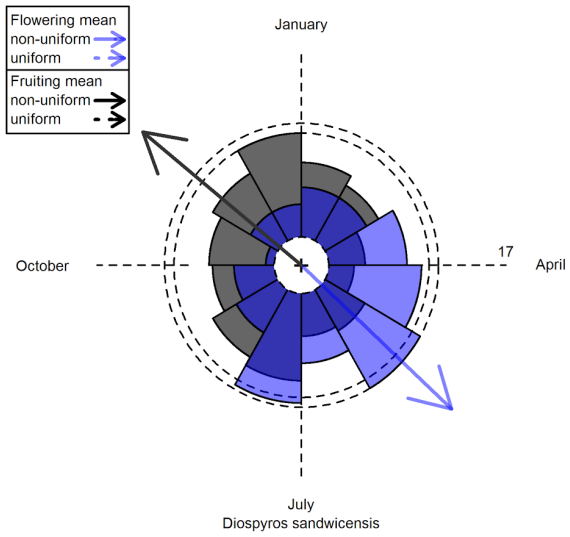
Coprosma foliosa



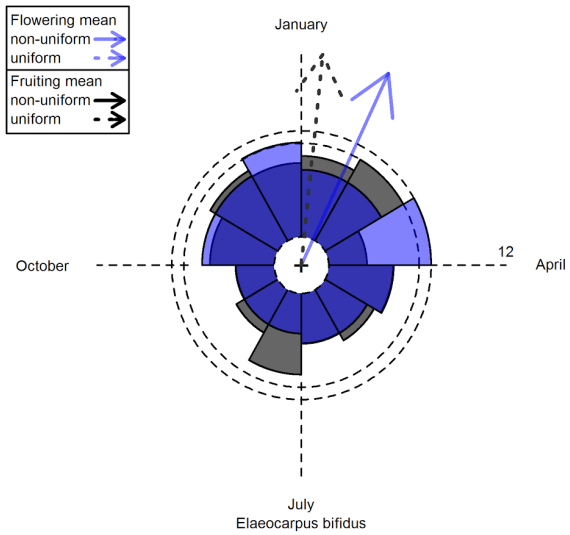
Coprosma longifolia



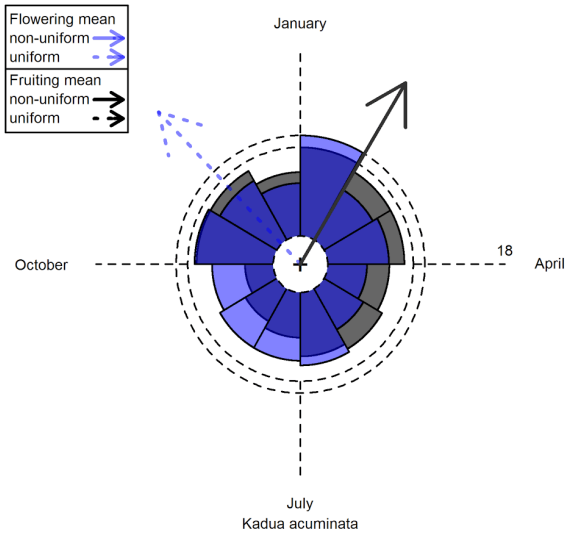
Coprosma ochracea



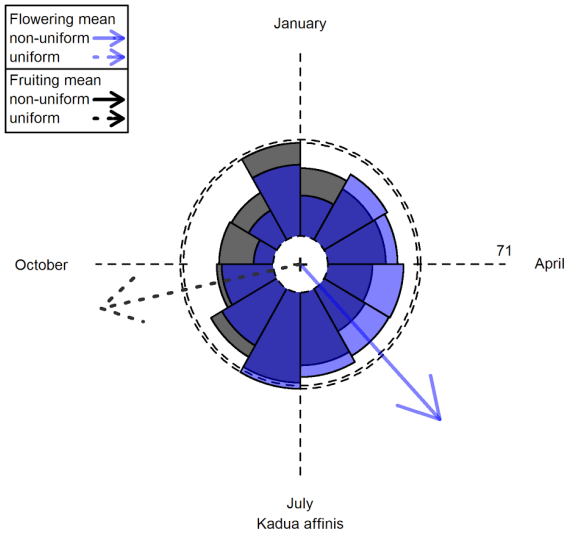
Diospyros sandwicensis



Elaeocarpus bifidus



Kadua acuminata



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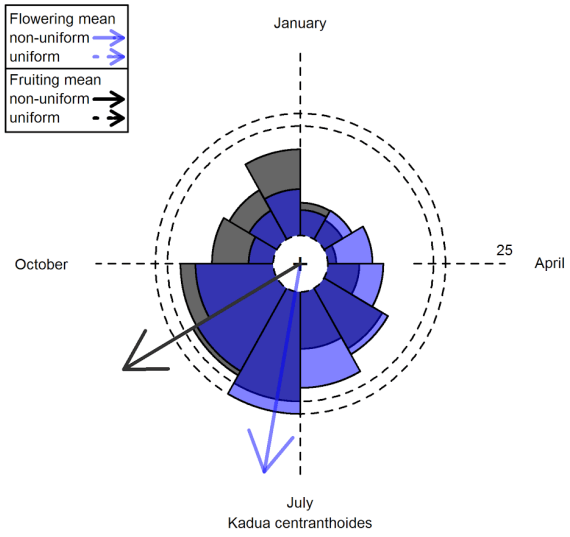

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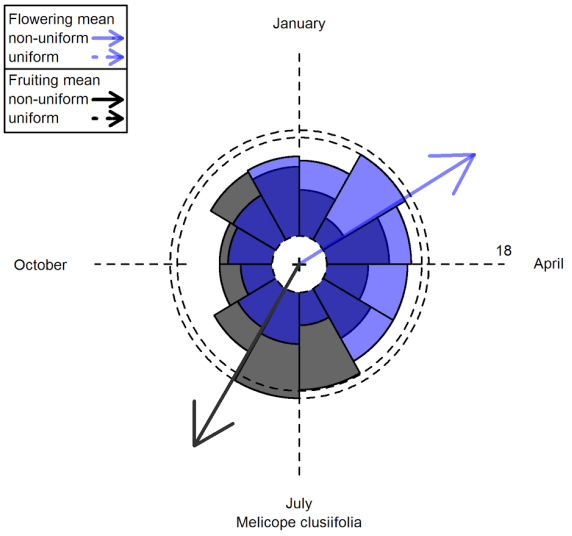

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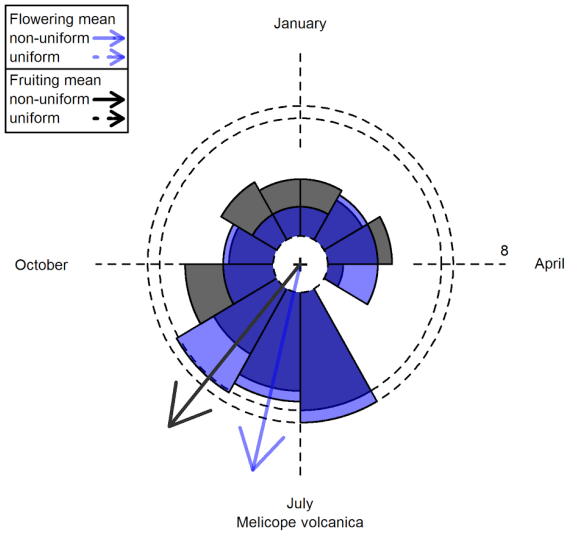

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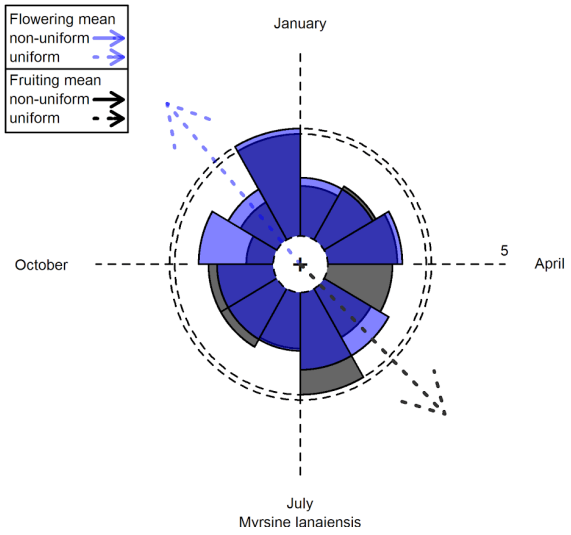

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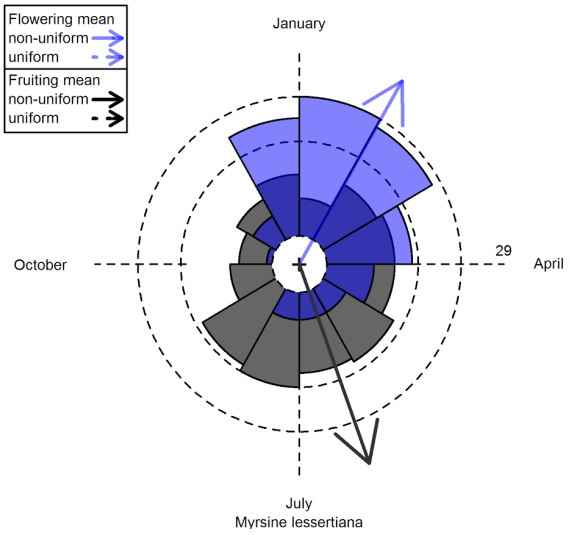

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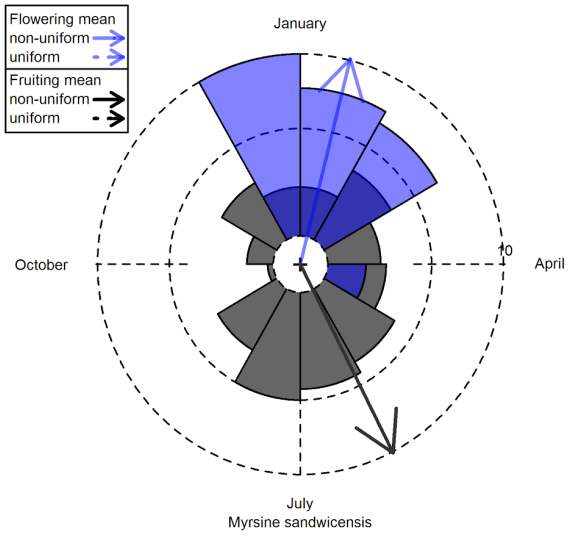

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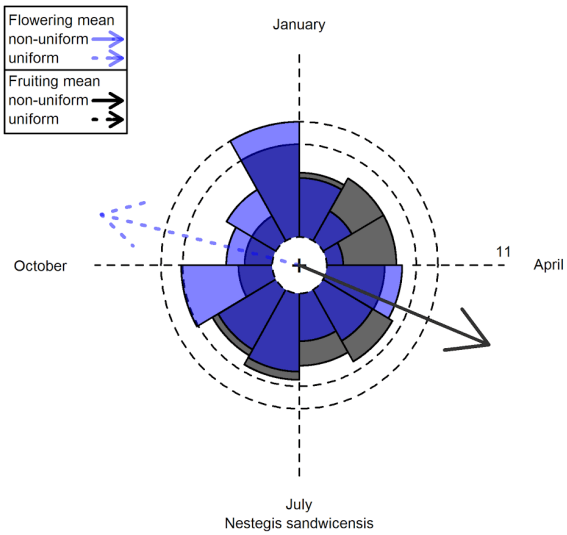

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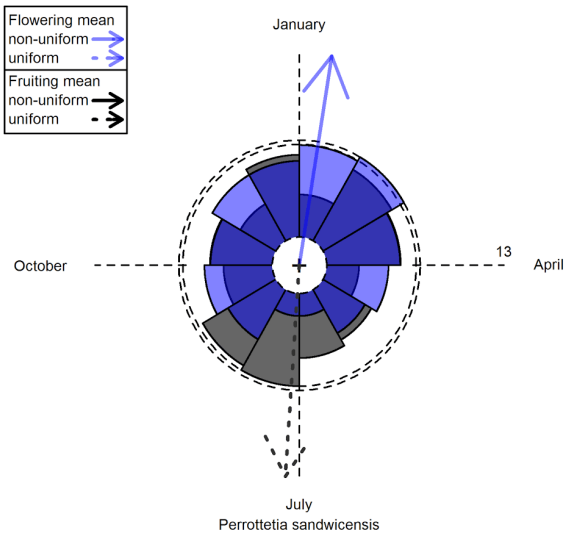

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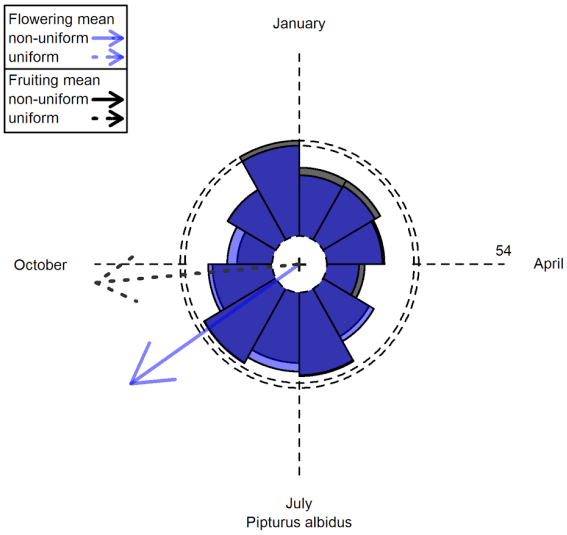

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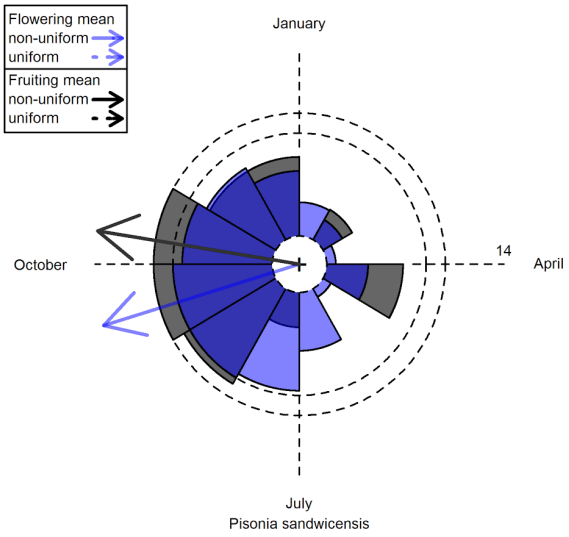

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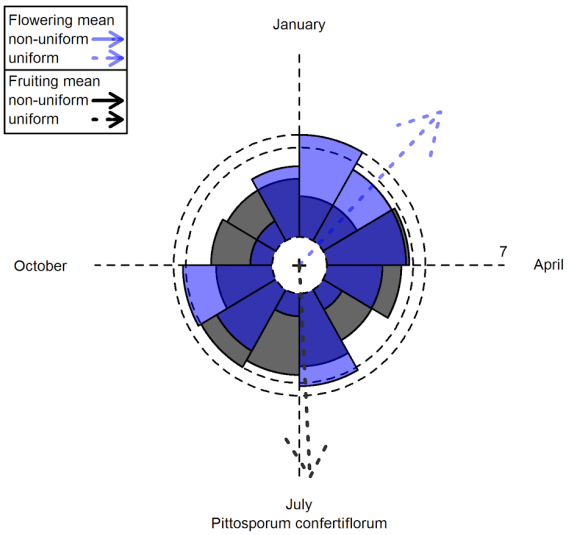

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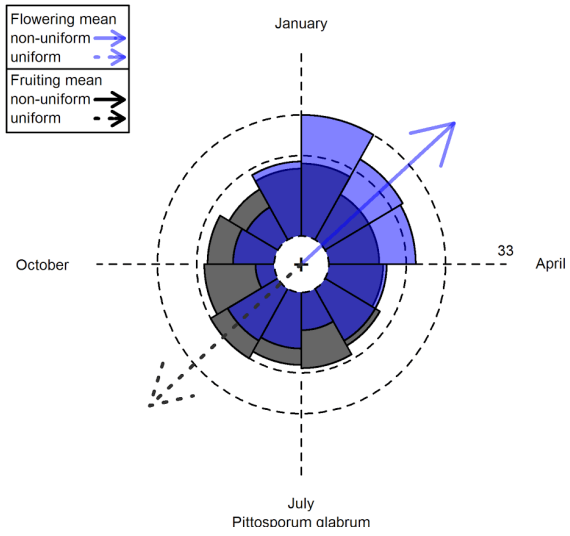

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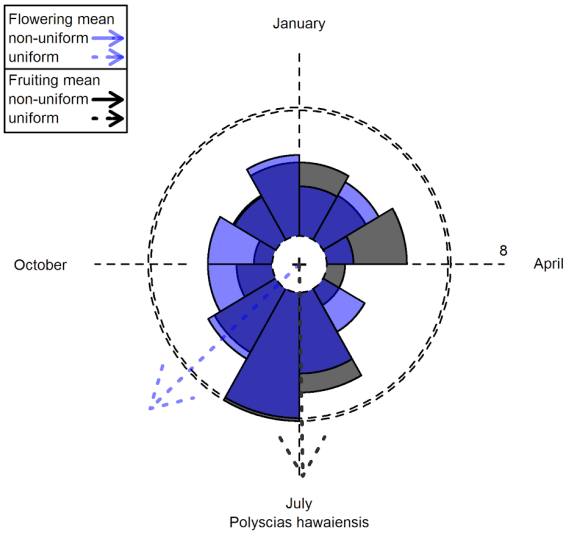

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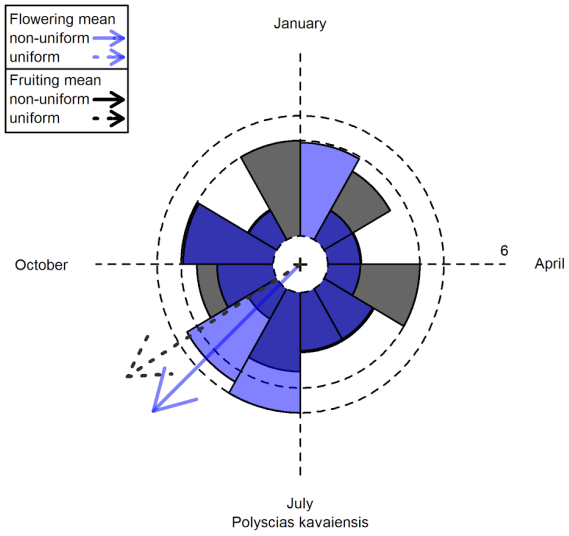

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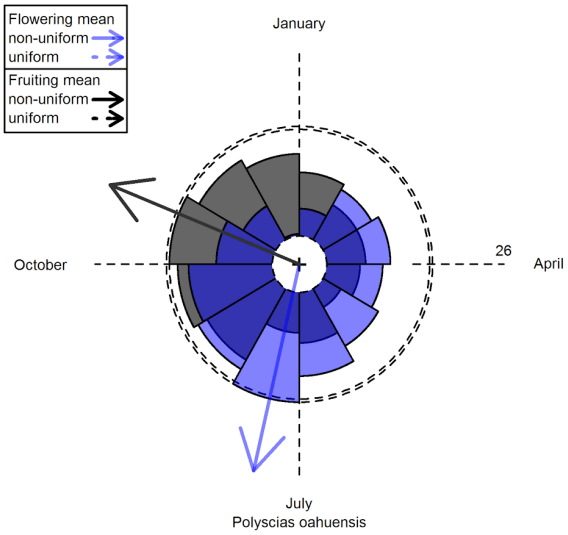

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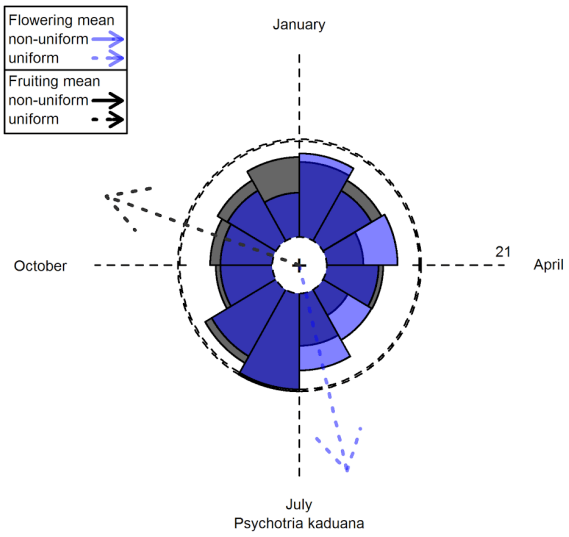

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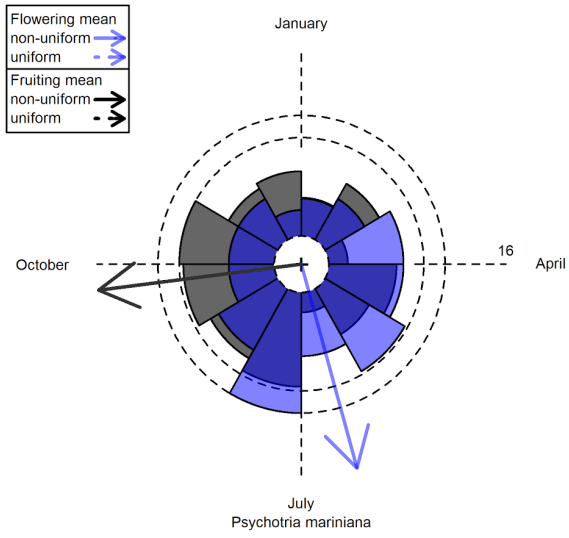

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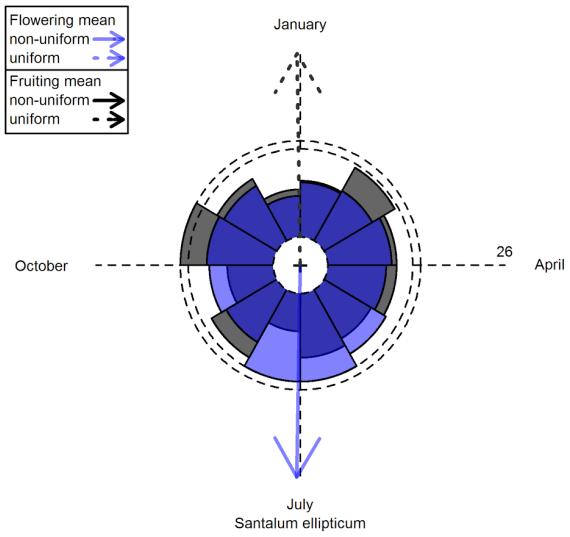

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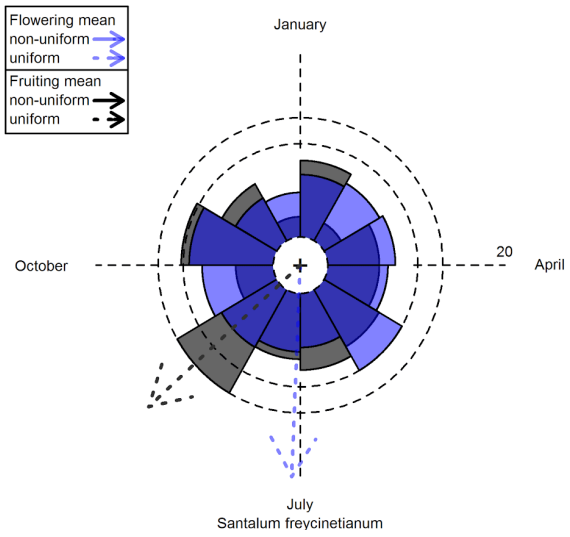

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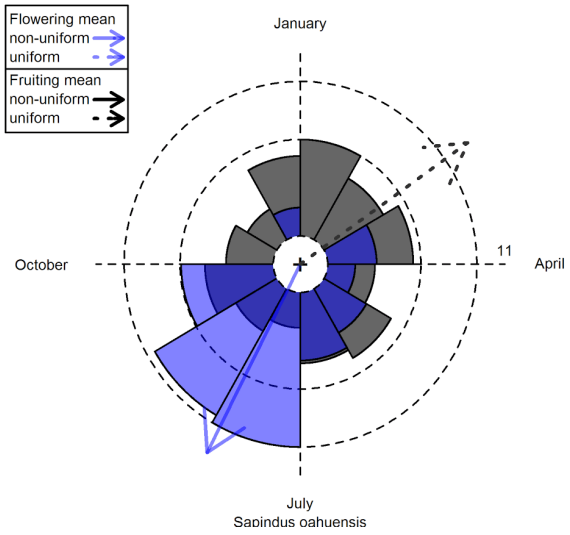

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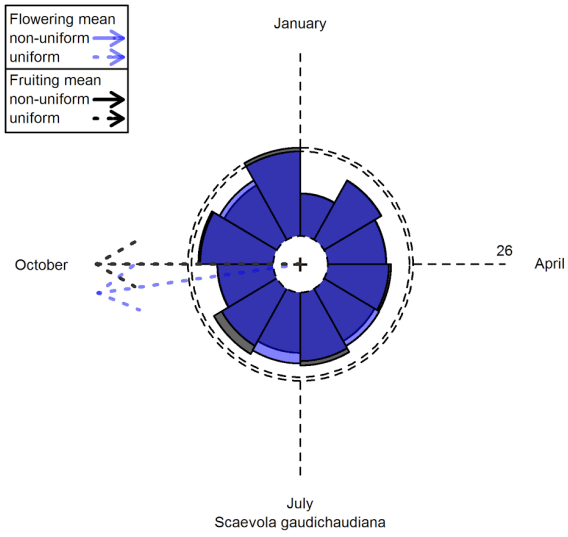

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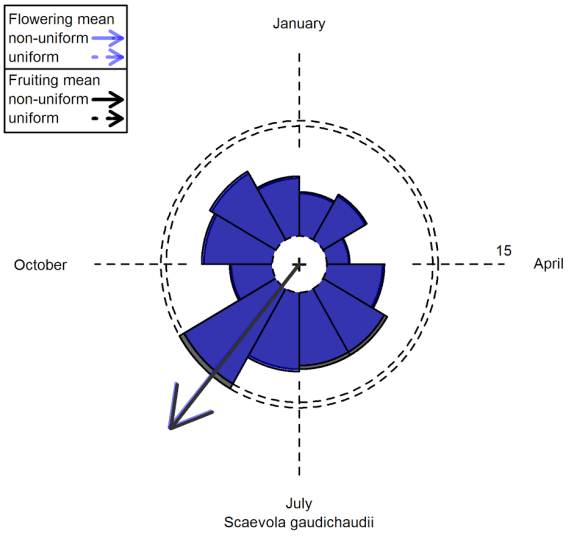

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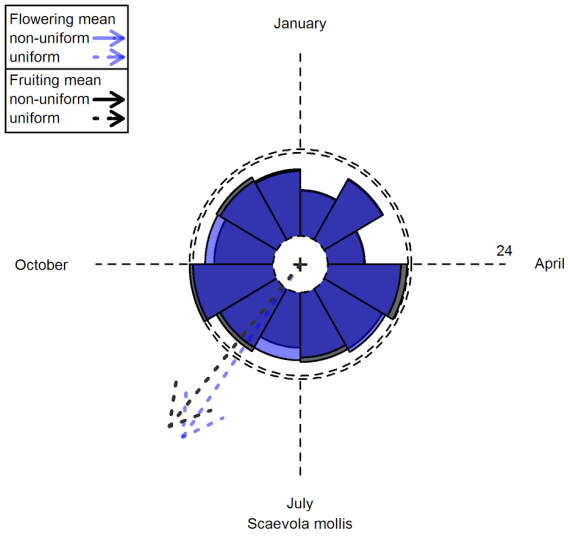

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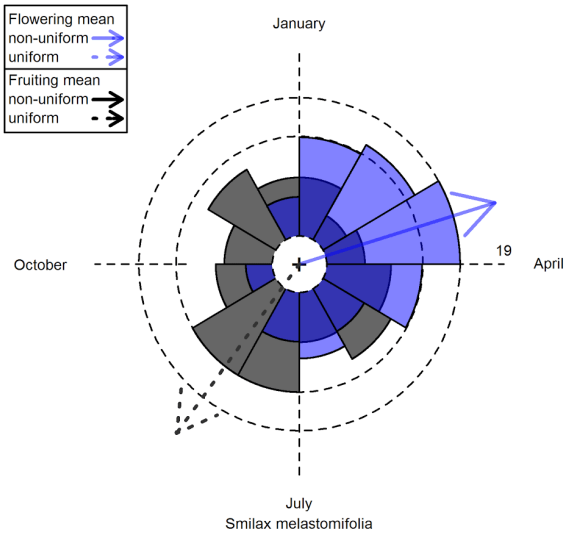

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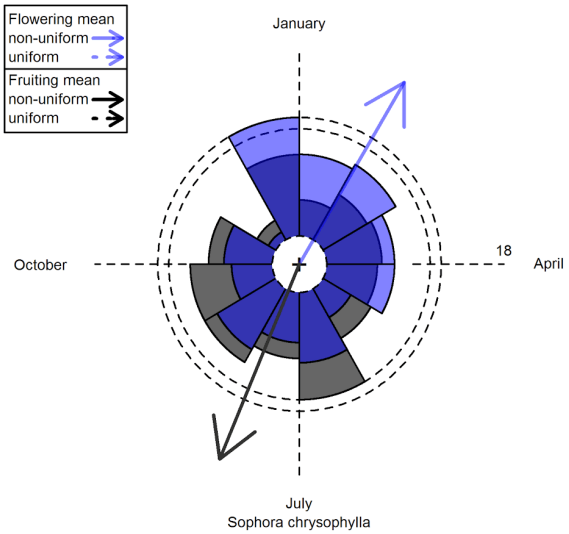

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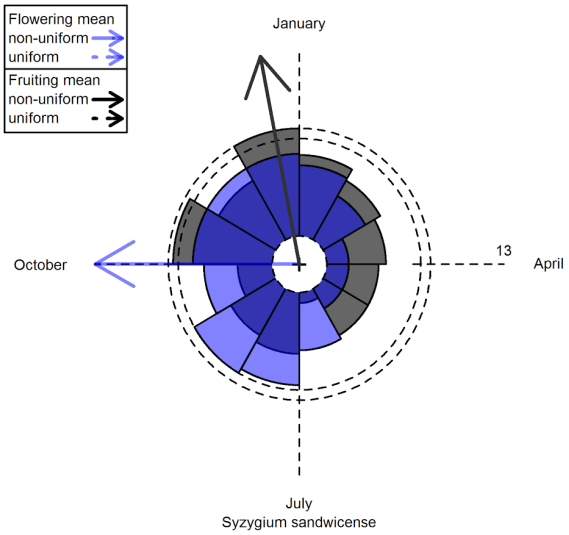

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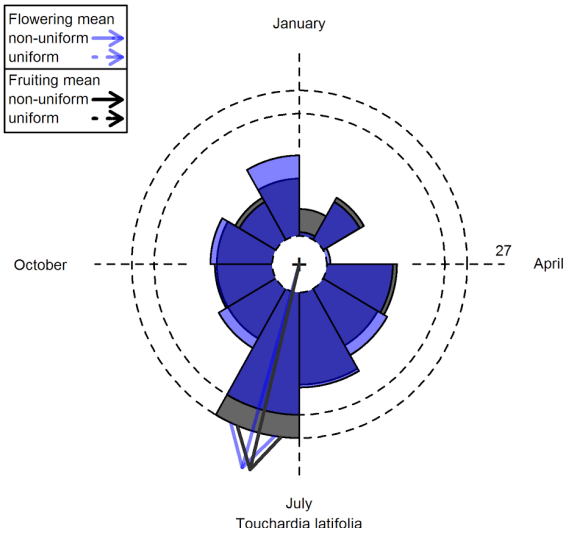

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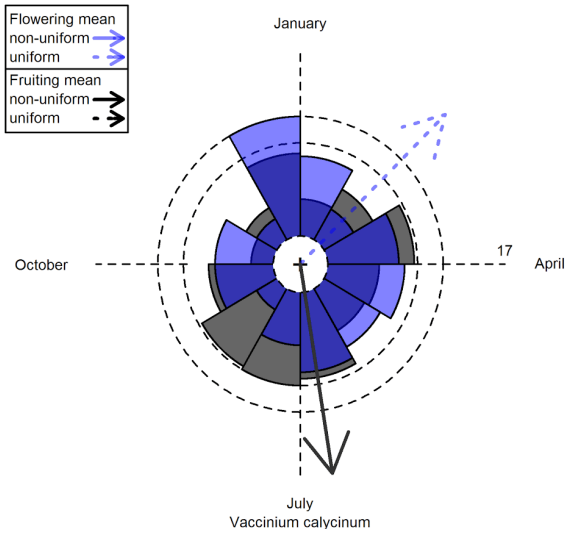

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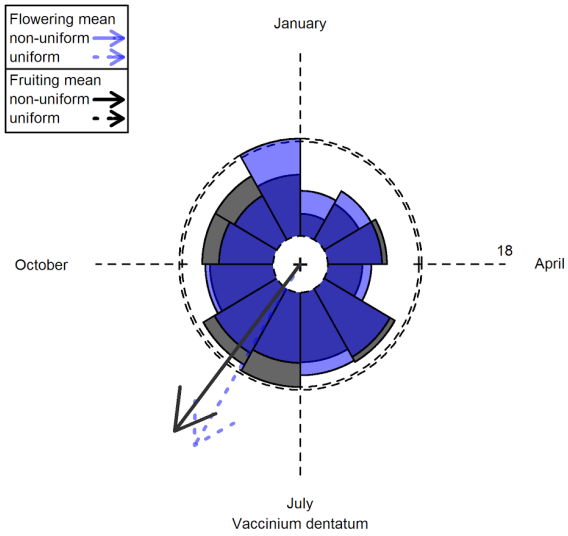

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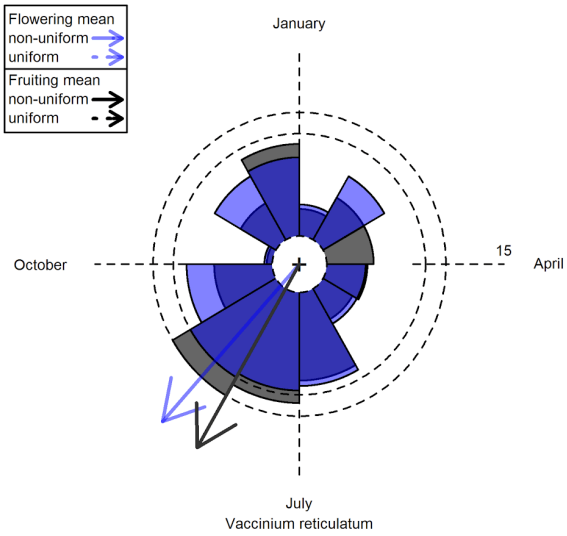

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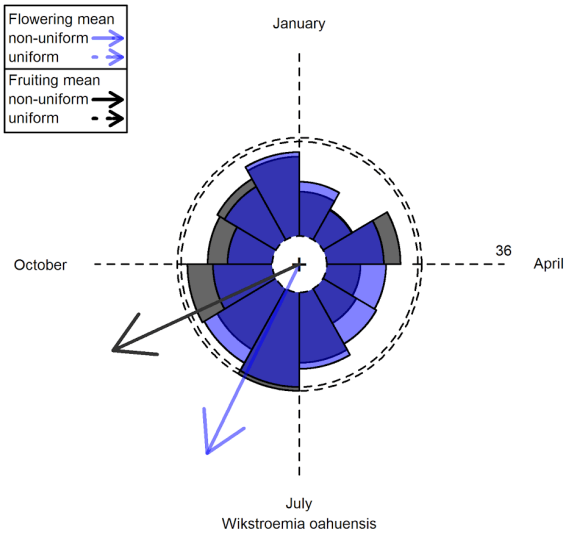

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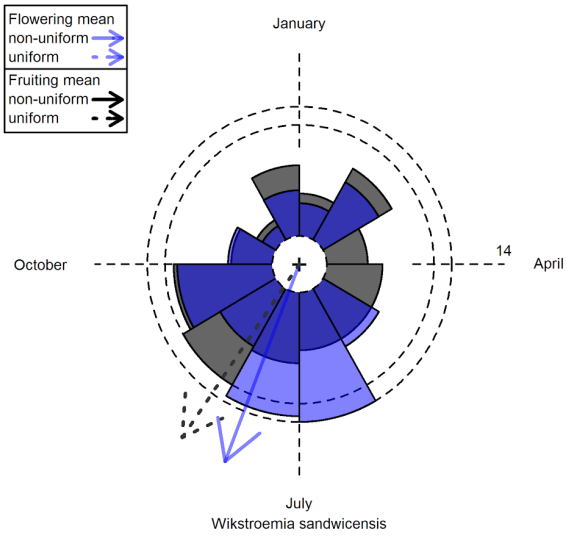

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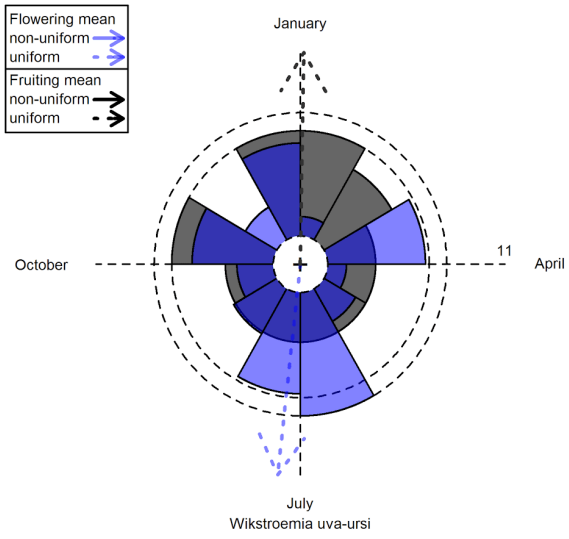

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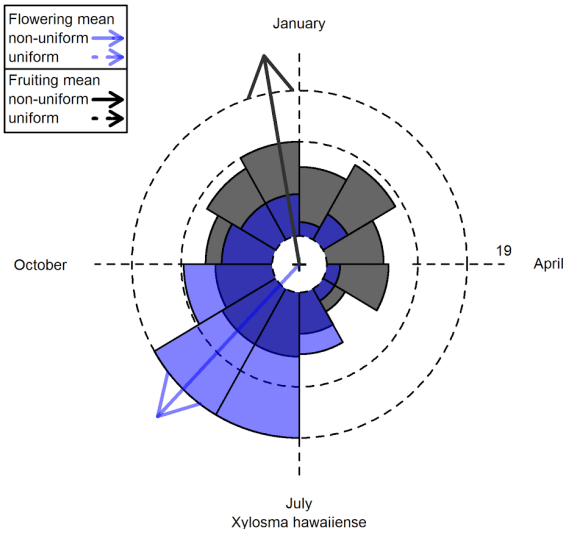

Xylosma hawaiiense

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