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The evolution of flowering phenology: an example from the wind-pollinated African Restionaceae

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• **Background and Aims** Flowering phenology is arguably the most striking angiosperm phenophase. Although the response of species to climate change and the environmental correlates of the communities have received much attention, the interspecific evolution of flowering phenology has hardly been investigated. I explored this in the wind-pollinated dioecious Restionaceae (restios) of the hyperdiverse Cape flora, to disentangle the effects of phylogeny, traits, and biotic and abiotic environments on flowering time shifts.
• **Methods** I recorded the flowering times of 347 of the 351 species, mapped these over a 98 % complete phylogeny and inferred the evolutionary pattern and abiotic correlates of flowering time shifts. The patterns and biotic/abiotic correlates of restio community mean flowering time were explored using 934 plots.
• **Key Results** Restios flower throughout the year, with large spring and smaller autumn peaks. Species flowering time is evolutionarily labile, poorly explained by either the environment or traits of the species, with half of all sister species allochronic. Community mean flowering time is related to elevation, temperature and rainfall.
• **Conclusions** Flowering time shifts may result from assortative mating and allochronic speciation, possibly leading to non-adaptive radiation. However, community mean flowering time may be environmentally selected. Diversification of flowering time may be non-adaptive, but species could be filtered through survival in suitable communities.

Key words: Allochronic speciation, Cape flora, flowering time, phenological niche, phenology, Restionaceae.

INTRODUCTION

Flowering is possibly the most striking aspect of the overall phenological niche in plants. In recent decades, it has received much attention both as a measure of climate change and to understand consequences of global change for plants and ecosystems (Fitter and Fitter, 2002; Parmesan and Yohe, 2003; Cook *et al.*, 2012; Wolkovich *et al.*, 2012, 2014; Flynn and Wolkovich, 2018). Flowering is a surprisingly complex phenomenon, with first flowering, last flowering and flower duration responding to different cues (CaraDonna and Inouye, 2015). Because flowering integrates both abiotic and biotic elements of the species niche (Pau *et al.*, 2011; Wolkovich *et al.*, 2014), it has even been proposed to be a summary of the environmental niche of the species (Forrest and Miller-Rushing, 2010). By shifting their flowering patterns, plants can avoid unfavourable times of the year for pollination, fruit set, seed release and seedling germination (Rathcke and Lacey, 1985; van Schaik *et al.*, 1993; Oberrath and Böhning-Gaese, 2002). However, although community flowering has received much attention, the patterns and processes underlying the evolution of flowering times, and the relative importance of the diverse factors acting on these, have not been investigated for any large clade at species level.

Theory predicts that flowering should occur when plants have been able to accumulate sufficient resources and when pollination will be efficient in order to maximize seed production, while leaving enough time to develop and release seed into optimal habitats under optimal conditions (Wolkovich *et al.*, 2014; Segrestin *et al.*, 2018). The flowering phenological niche (Gotelli and Graves, 1996; Chuine, 2010) thus represents a complex balancing act for plants, involving a set of compromises between vegetative growth, seed development time and biotic interactions, in the context of the local climate (Bolmgren and Cowan, 2008) and the biotic community. Phenological patterns are further influenced by seasonal fluctuations in limiting factors (Boulter *et al.*, 2006). Because optimal phenology maximizes seed production and quality, it improves Darwinian fitness directly, and may therefore be a trait that is under strong selection (Wright and Calderon, 1995). Among the most important seasonally fluctuating limiting factors are probably water availability, temperature, and biotic factors such as availability of pollinators and seed dispersal agents. Water availability on well-drained soils is driven by rainfall, and modulated by the rooting depth of the plants (Cortes-Flores *et al.*, 2017), but in wetlands water availability may be decoupled from rainfall (de Carvalho *et al.*, 2015). Temperature may be important both as a measure of a period of physiological activity and by modulating transpiration rates. Biotic factors may influence flowering time via facilitation, where synchronized flowering enhances pollinator attraction (Rathcke and Lacey, 1985; Sargent and Ackerly, 2008); 'diffuse facilitation' (Sargent and Ackerly, 2008; Cortes-Flores *et al.*, 2017), where scattered and staggered flowering of woody trees during the dry season maintains a food resource for pollinators; and competition (Robertson, 1895;

Armbruster *et al.*, 1994), where flowering times are shifted to reduce competition for pollinators.

Most researchers report a phylogenetic pattern for at least some components of the phenological niche. However, these are all studies of regional floras (e.g. Johnson, 1993; Davies *et al.*, 2013; Du *et al.*, 2015), or of local sample sites (e.g. Kochmer and Handel, 1986; Willis *et al.*, 2008; Mazer *et al.*, 2013; Cortes-Flores *et al.*, 2017; Schneider, 2017), using a phylogeny or classification resolved to family or at most generic, but not species, level. Consequently, these studies demonstrate phylogenetic phenological niche conservatism at family, and not necessarily at species, level (Levin, 2006), and demonstrate that some families tend to flower earlier than others. This has been referred to as 'phylogenetic temporal niche conservatism' by Du *et al.* (2015). However, it is unclear whether there is phylogenetic phenological niche conservatism at species level. Flowering time shifts between closely related species have been demonstrated in many plant groups, and have repeatedly been shown to contribute substantially to the prezygotic barriers to gene flow – largely because these act first in the sequence of isolation barriers [e.g. in *Roscoea* (Zingiberaceae; Paudel *et al.*, 2018), *Mimulus* (Phrymaceae; Ramsey *et al.*, 2003) and Howieae (Arecaceae; Savolainen *et al.*, 2006)]. At least some phenological shifts have been shown to be genetically controlled (Hipperson *et al.*, 2016). Such flowering time shifts may lead to assortative mating (Elzinga *et al.*, 2007; Weis *et al.*, 2014), as in Louisiana irises (Cruzan and Arnold, 1994), Spanish oaks (Goicoechea *et al.*, 2015) and South African mesembs (Ellis *et al.*, 2006). This pattern of phenological divergence among closely related species is more suggestive of evolutionary lability than of phylogenetic conservatism.

Flowering time shifts may also be linked to habitat changes (Savolainen *et al.*, 2006) and, as such, assortative mating could facilitate specialization to these habitats (Paudel *et al.*, 2018). A variable environment leading to phenological displacement, resulting in assortative mating driving genetic differentiation, has been described as the 'Asynchrony of Seasons Hypothesis' (Martin *et al.*, 2009). Flowering time shifts in *Agrostis tenuis* and *Anthoxanthum odoratum* on and off mine tailings are likely to be associated with reduced gene flow between the two ecotypes, and may have led to the differentiation of the ecotypes, and the start of a speciation process (McNeilly and Antonovics, 1968). Analyses above the species level have provided mixed results: speciation may be associated with flowering time shifts (van der Niet and Johnson, 2009; Pace *et al.*, 2019) or not (Perret *et al.*, 2007). A special case of speciation associated with phenological shifts is allochronic speciation, where speciation is driven directly by phenological divergence, and not by environmental variables or habitat changes (Yamamoto and Sota, 2009; Taylor and Friesen, 2017).

Almost no studies have explored the pathways by which evolutionary shifts in flowering phenology occurred in plants, and hence evaluated the balance between adaptation to the environment, inherited flowering constraints and shifts associated with speciation. The possibility that phenological shifts may be fixed by assortative mating, and be transformed from an ecological to a phylogenetic pattern by allochronic speciation, has not been investigated empirically, although Robertson (1895) did hint at this 120 years ago. Here, I use the wind-pollinated dioecious Restionaceae in the South African Cape flora to explore flowering time evolution. Restionaceae are widespread in the Southern Hemisphere, with just one species in the Northern Hemisphere. There is one species in South America (Chile), four in New Zealand, 150 in Australia, one widespread in South-east Asia and 350 in Africa. All species are perennial, evergreen, grass-like plants, with annual flowering (*sensu* Newstrom *et al.*, 1994), and almost all are dioecious. The African Restionaceae (subfamily Restionoideae, hereafter referred to as 'restios') are monophyletic, and embedded in the rest of the family. Restios are almost restricted to the Cape flora of southern Africa, where they are common to locally dominant elements of the fynbos vegetation, typical of the oligotrophic sandstone-derived soils (Rebelo *et al.*, 2006). The earliest southern African fossils are from the Palaeocene (Scholtz, 1985); most of the diversification occurred during the Neogene (Bouchenak-Khelladi and Linder, 2017). The Cape restios are an ideal group for exploring flowering time evolution, as flowering can occur, depending on the species, at any time of the year, allowing a great evolutionary flexibility. Flowering throughout the year may be possible because the Cape, like other Mediterranean systems, is unusual in having two optimal growing seasons with both water and warmth, separated by two sub-optimal but not lethal seasons: a hot dry summer and a cold wet winter. Moreover, all restios are wind pollinated, which removes the confounding effects of plant–pollinator interactions.

I used the following pipeline to explore the patterns and processes in the evolution of the restio flowering times. I first ask (1) whether restio communities differ in flowering time, and whether this difference can be explained environmentally (correlated with temperature, elevation, rainfall patterns or groundwater availability). Having established that there are differences in community peak flowering time, I (2) explore the evolution of flowering time differences among restio species. I test two process explanations. First (2a) that flowering time shifts are associated with speciation, so that sister species are frequently allochronic. To evaluate the significance of allochronic speciation, I compare its frequency with that of allopatric speciation. Secondly (2b) that flowering time shifts are due to adaptation to environmental variables (temperature and water availability).

MATERIALS AND METHODS

Datasets

Flowering time. Species flowering times were compiled from field notes – largely my own observations over the past 25 years – recording the presence of fresh anthers or stigmas, and approx. 12 000 herbarium specimens primarily from the Bolus Herbarium (BOL) and Compton Herbarium (NBG) (Supplementary data Table S1). Flowering time data are available for all but four of the 351 species from the Greater Cape Floristic Region (GCFR). Flowering assessed in the field is unambiguous, but on herbarium material care needs to be taken to check that the anthers or stigmas were fresh when collected. Because old spikelets are persistent and many species are easier to identify when fruiting rather than flowering, they are collected throughout the year. The flowering data were

collected at a resolution of 1 month. The data were recorded by species rather than collection location; consequently, there are no data on the spatial variation in flowering times. However, intraspecific spatial variation may be important (Craine *et al.*, 2012*a*, *b*; Spriggs *et al.*, 2019), and may have a genetic basis (Olsson and Agren, 2002; Stinchcombe *et al.*, 2004), and accounting for it may give the data more resolution. Although spatial undersampling may result in misleading interpretations if the flowering dates are precise (de Keyzer *et al.*, 2017) due to false absences, pooled flowering data will have fewer false absence data, but err in losing resolution. Consequently, any signal obtained from such crude data is likely to be robust. Opportunistic observations on several species of restios indicate that the stigmas of each flower remain receptive for several days, but that pollen release occurs mainly near sunrise; however, this has not been formally tested. There is, nevertheless, no indication that species may be separated by diurnal flowering patterns.

Plot data. Community phenology was inferred from 937 plots across the GCFR (Fig. 1). The plots (available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.c8v5630) are circular and 10 m in diameter, and were placed subjectively to include all habitats, climatic zones and geographical regions in which restios are found in the GCFR, and as far as possible also all restio species. For each plot, the restio species present were listed, basic habitat descriptors noted (bedrock, soil depth, soil drainage, slope and aspect) and GPS co-ordinates recorded. Many of the plots were established along transects across the major mountain ranges, and all possible restio habitats were sampled, from sea level to many of the highest summits, up to 2250 m. Three plots missing critical observations were excluded, leaving 934 plots in the study. No phenological data were collected directly, but were inferred by combining the species lists of each plot with the flowering months of the species. From this combined file, I calculated for each plot (1) the number of restio species with flowering information; (2) the number of species flowering in each month; (3) the month in which most species flower, and how many months share this number; and (4) the number of months during which at least one species flowers (Supplementary data Table S2).

Environmental and trait data. The climate data for each species were inferred from an occurrence dataset of 12 933 records compiled primarily from georeferenced herbarium specimens and plot data, with both taxonomy and locality data carefully revised (available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.c8v5630). For each record, the CHELSA climatology (Karger *et al.*, 2017), downloaded in January 2019, was used to document mean annual precipitation (MAP), rainfall seasonality and rainfall in the driest quarter (as a measure of the severity of seasonality), the month with the highest rainfall (in order to quantify the shift in rainfall season), mean annual temperature (MAT) and, as measures of temperature fluctuation, the variance of the temperatures and the isothermality. These variables were chosen to reflect climate variation across the Cape, which ranges from summer drought in the west to all-year rainfall in the east; from <300 mm per year in the dry

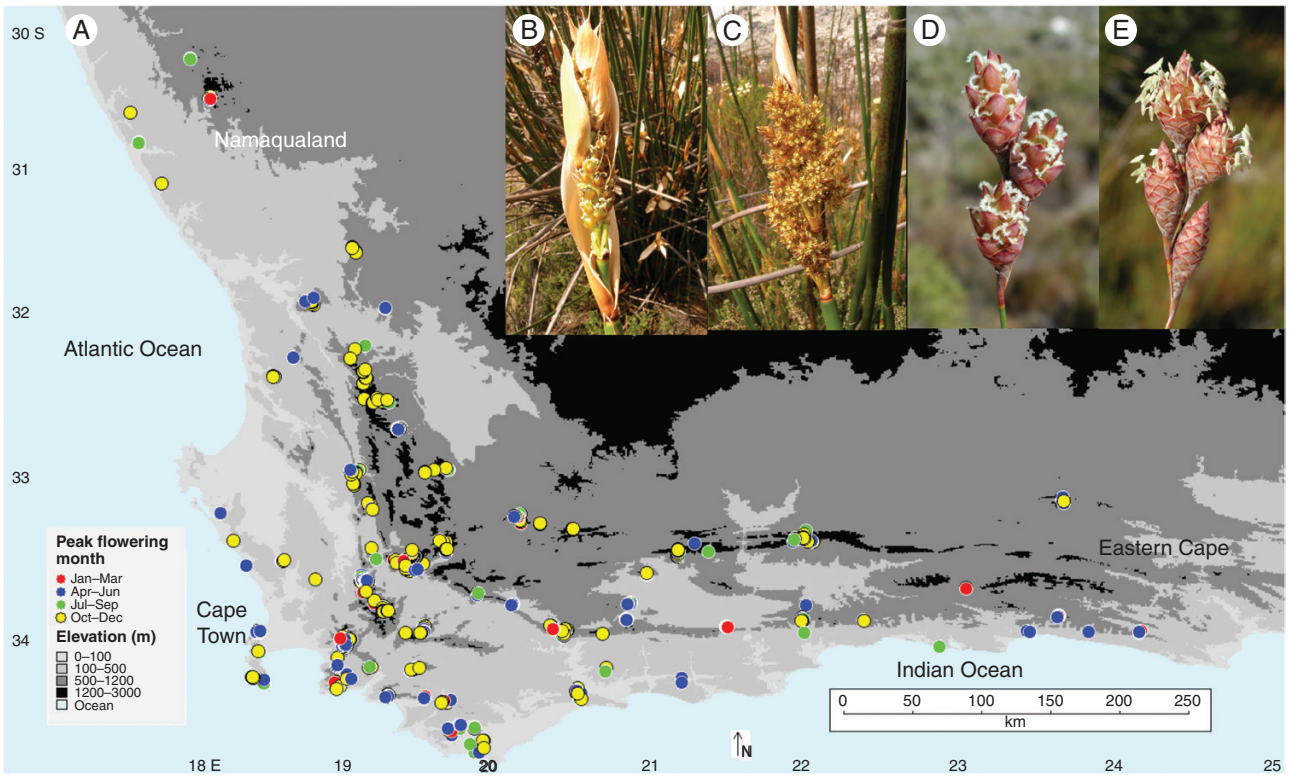

FIG. 1. (A) Geographical distribution of plots used to infer spatial, climatic and ecological drivers of flowering time. The plots are colour coded by community peak flowering month: red – January and February; blue – March to May; green – June to August; yellow – September to December. Many plots are spatially clustered; consequently the dots overlap. (B) Female *Elegia mucronata*, (C) male *E. mucronata*, (D) female *Restio strobilifer*, (E) male *R. strobilifer.*

inland valleys to >3 m on the highest SW peaks, and from an oceanic climate along the coast to a more continental climate along the inland margins of the region. The data were simplified to the median value for each species, as this should reduce the impact of geographically and climatically biased sampling (Supplementary data Table S1). Isothermality and rainfall in the driest quarter were not included in subsequent analyses as they are correlated with variance in the temperature and MAP.

Dispersal mode was the only plant trait included in the analyses, as dispersal mode has been shown by Oberrath and Böhning-Gaese (2002) to influence flowering time. Dispersal in restios can be categorized as wind dispersal, ballistochorous dispersal and ant dispersal (Linder, 1991); the data were taken from Linder (2001).

Phylogeny. The phylogeny was published in Linder (2019) (trees available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.c8v5630), and includes 346 of the 351 restio species and infraspecific taxa (all referred to here as species) from the GCFR. I retained a single tip per species, and removed those species without flowering data, leaving 338 species in the analysis. The phylogeny, based only on the chloroplast loci *atpB–rbcL*, *trnK–matK*, *trnL–F*, *psbA–trnH* and *rpl32–trnL*, was built using maximum likelihood implemented in RaxML 8 (Stamatakis, 2014), rate corrected using BEAST (Drummond and Rambaut, 2007) and time calibrated using a Palaeogene fossil pollen occurrence in South Africa and extant limestone endemic species from the South African Agulhas Plain (Bouchenak-Khelladi and Linder, 2017). The topology is consistent with previous analyses (Bouchenak-Khelladi and Linder, 2017). All downstream analyses were done with the maximum clade credibility (MCC) tree.

Analyses

Community flowering times. In order to test whether there are differences in the flowering times among restio communities (here taken to be the plots), and to test their correlates, I calculated the community peak flowering month, taken as the month in which most restio species flowered. For plots with several months with the same maximal number of species flowering, a month was randomly selected. In order to test whether this influenced the results, sampling was repeated 100 times, and the significance of the differences in community peak flowering was tested using an analysis of variance (ANOVA).

Testing correlates of community peak flowering is problematic. Flowering time is circular (Pewsey *et al.*, 2013) and, although effective Bayesian regression approaches that use linear predictors for a circular response variable exist, these assume a von Mises distribution, which is essentially a normal distribution (Mulder and Klugkist, 2017), and the data here are bimodal. Consequently, I used correlation tests implemented in the R (R Development Core Team, 2017) package 'circular' (Agostinelli and Lund, 2017) employing the script suggested by Pewsey *et al.* (2013). Geographical, climatic and habitat correlates of flowering time were explored separately. Geography, and so distance between the plots (which can also be interpreted as a measure of spatial autocorrelation), was quantified by latitude and longitude. Climatic variables both have a strong spatial pattern and also co-vary, and so cannot be assumed to be independent of each other or of the distance between the plots. I used a principal components analysis calculated from ranged and centered climatic data to extract independent (orthogonal) axes. The principal components were correlated with flowering time as well as latitude and longitude. Habitat similarity was measured as ground water availability. This was simplified to well-drained vs. wetland habitats, and the proportion of well-drained vs. wetland plots flowering each month was tested with a χ^2 test, against the null hypothesis that this proportion stays the same throughout the year.

Evolution of flowering time. The ancestral flowering time, and evolutionary shifts in flowering time, were determined by optimizing flowering time on the phylogeny for the 338 species with flowering time and included in the phylogeny. Flowering phenology data are complex, and can be simplified into five measures. (1) First flowering date is especially suitable for documenting flowering time shifts in response to climate change (e.g. Fitter and Fitter, 2002). However, this may be biased by population size and sampling frequency (Miller-Rushing *et al.*, 2008), and is less useful for documenting flowering time across a clade because rarely sampled or rare species could have an artificially delayed first flowering compared with frequently sampled or common species. (2) Last flowering, like first flowering, is also sensitive to sampling (CaraDonna *et al.*, 2014), and is almost never used. (3) Peak flowering data (i.e. when most plants flower) requires frequency data, i.e. populations or individuals of a species, and this information is not available for restio species (but is for communities; see above). (4) Duration of flowering, treated by Kochmer and Handel (1986) and Wright and Calderon (1995) as the number of months of flowering, is also susceptible to undersampling effects. (5) Mid-range flowering (halfway between first and last flowering), which is often used (Kochmer and Handel, 1986; Wright and Calderon, 1995; Bolmgren *et al.*, 2003; Warren *et al.*, 2011; CaraDonna and Inouye, 2015; Du *et al.*, 2015; Schneider, 2017), can be considered a conservative measure least influenced by mistaken first and last flowering dates caused by sampling biases. This measure was used here for the species analyses, as first and last flowering dates are the only information available.

In temperate regions, circular flowering time can be transformed into the days from end of winter to beginning of winter, or simply as the day of the year. In tropical regions, this problem is sometimes dealt with by transforming flowering time into a circular vector (Kochmer and Handel, 1986; Morellato *et al.*, 2010; Cortes-Flores *et al.*, 2017). To infer ancestral flowering time in the restios, and to test whether flowering time is phylogenetically conserved, polymorphism in flowering month and the circular time scale problems were solved by scoring each month as a state (thus 12 states) and weighting character state transitions using a stepmatrix in which each additional month change is weighted by 1 (Supplementary data Table S3), thus mimicking a circular character. Tracing character evolution across a tree using a stepmatrix and parsimony is implemented in Mesquite 3.51 (Maddison and Maddison, 2017). The disadvantage of this approach is that optimization can only be done using parsimony methods, thus ignoring variation in branch lengths, but the advantage is that there is no simplification of the data.

Flowering shift with speciation. To explore the evolution of flowering time, this was simplified to the mid-point of each range (mid-range flowering time). This was linearized as the deviation from the ancestral flowering time, which was inferred by parsimony optimization using a stepmatrix to be September–October, as the mid-point of the range (both months scored as 6), the start as April (scored as 1) and the end as March (scored as 11). Inspection of the optimized values on the phylogeny suggests that there are few cases where flowering in summer or late summer could best be interpreted as a shift to earlier flowering from the ancestral condition; in these, linearization will lead to incorrect weighting of flowering time shifts. The inferred mid-range linearized flowering times were ranged from 0 to 1. I first tested whether flowering time evolution was phylogenetically constrained by calculating Pagel's lambda (λ) (Pagel, 1999) and Blomberg's K (Blomberg *et al.*, 2003), and comparing these with null distributions generated from 10 000 simulations, as implemented in the R package 'phytools' (Revell, 2012). Then I explored whether flowering time shifts are more likely to be associated with speciation events than branch lengths. If so, then we expect that Pagel's kappa (κ) should approach zero (indicating that most shifts occur at speciation and not as a factor of branch length), and Pagel's delta (δ) should be higher than 1 (indicating that most changes occur near the tip of the tree; Pagel, 1999; Harmon, 2018). Furthermore, if flowering time changes frequently with speciation and so is evolutionarily labile, then the phylogenetic signal (compared with the Brownian motion and Ornstein–Uhlenbeck null models) should be insignificant. To determine the fit and parameterize the Brownian motion, λ, Ornstein–Uhlenbeck, δ and κ models, I used the function *fitContinuous* implemented in the R package 'geiger' (Harmon *et al.*, 2015).

Finally, I postulate that if rapid shifts, associated with speciation events, occur occasionally, followed by inheritance of flowering time, then removing these events should increase the estimated value of Pagel's λ. Consequently, I removed all 'shift species'; these are species in allochronous sister species pairs which showed no overlap with their ancestral flowering time (thus double allochronous: in comparison with the sister species, and in comparison with the ancestral node, as optimized using parsimony and a stepmatrix), and calculated Pagel's λ.

To test if allochronic shifts are associated with geographical range shifts (allopatry vs. sympatry) or habitat shifts, and to compare their frequencies, these were contrasted in sister species. Geographical ranges were taken from range polygons based on all occurrence data, with overlapping ranges classified as sympatric and non-overlapping ranges as allopatric. Habitat shifts were separated into climatic and edaphic shifts. Climatic shifts were quantified as the sum of the differences in mean annual precipitation, rainfall seasonality, dry quarter rainfall and minimum elevation. In order to make all four variables equivalent, the data were ranged from 0 to 1. Edaphic shifts were simplified to four ground water conditions [permanently wet (marshes, streambanks), seasonal seepages, habitats with shallow groundwater and well-drained habitats] treated as a meristic character (Supplementary data Table S4). The frequencies of geographical, climatic and ground water shifts were compared between allochronous and synchronous sister species. In order to test for phylogenetic uncertainty, all analyses were repeated for sister species with posterior probabilities of >0.8, 0.9 and 0.95.

Flowering time shifts with environmental adaptation. In order to test whether any environmental variables are associated with shifts in flowering time, I used phylogenetically corrected regressions implemented in phylolm, in the R package 'phylolm' (Ho and Ane, 2014), using the linearized median flowering times, the MCC tree and λ to estimate the errors. The pseudo R^2 was calculated with the R package 'RR2' (Ives and Li, 2018), as this can take into account the lack of independence in the error distributions.

Three datasets were analysed. The first is the complete dataset. Secondly, in order to determine whether species which are buffered against the climate [i.e. growing in wetlands, in areas with low seasonality or with very long flowering times (>3 months)] 'dilute' the climate signal, these species were removed from the dataset. If they do, then removing them should reduce the pseudo R^2 and the significance of the regression coefficient. The third dataset, with the 50 'shift species' removed, tests whether dramatic shifts in flowering time might be associated with speciation rather than adaptation to the environment. Removing these species should increase the pseudo R^2 and the significance of the regression coefficient.

RESULTS

Community flowering time

Community flowering duration is 8.6 ± 3.2 months. Overall peak flowering is in late spring, in October, with a smaller second peak in late autumn, in April. The two low points are early winter (June–July) and mid-summer (January) (Fig. 2). The community peak flowering month shows a weak but statistically significant geographical pattern,

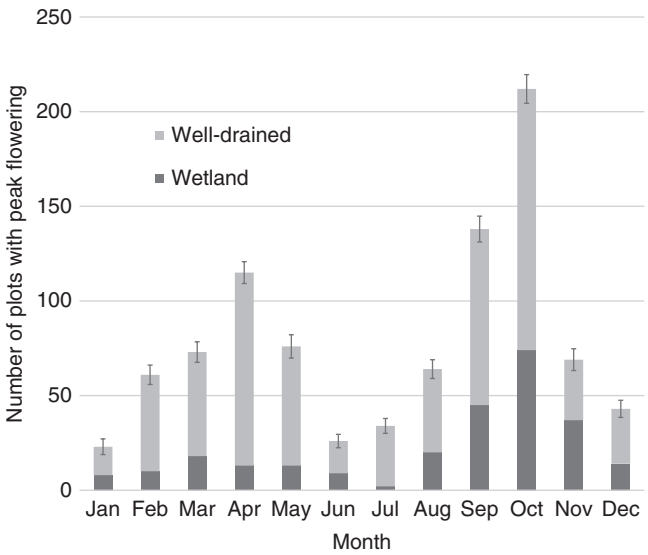

FIG. 2. Community peak flowering distributed through the year, with wetland (black) and well-drained (grey) plots separated. The error bars reflect 100 random samples from the plots with more than 1 month of community peak flowering; the monthly distribution differs significantly (ANOVA: d.f. = 1, $F = 122.9$, $P < 0.001$). The monthly distribution of community peak flowering also differs significantly between wetland and well-drained habitats ($\chi^2 = 54.495$, d.f. = 11, $P < 0.001$).

flowering marginally earlier in the west compared with the east ($R^2_{x\theta}$ = 0.02, $P < 0.001$) and earlier in the north (i.e. inland) compared with the south ($R^2_{x\theta}$ = 0.04, $P < 0.001$). This is also evident in Fig. 1.

The principal components (Fig. 3) effectively summarize the climatic data: PC1 reflects the elevation gradient and associated decrease in mean annual temperature and increase in temperature variance; PC2 the mean annual precipitation gradient; PC3 rainfall seasonality; and PC4 the month with maximum rainfall (Table 1). These four eigenvectors account for 95.8 % of the total climatic variation. The relationship to geography is weak, but fits the broad picture: rainfall seasonality relates to longitude, with high seasonality in the west (summer drought) and low in the east (all-year rainfall). Latitude is related, albeit weakly, to both the rainfall gradient (wet coast to dry inland) and the elevation/temperature gradient (oceanic coast to continental inland). However, the low variation explained is presumably due to the arid intermontane valleys alternating with colder, wetter east–west trending mountains. Although community peak flowering month is significantly related to all four eigenvectors, all correlations are very weak. PC1 shows that at higher elevations and with lower temperatures, flowering is significantly more frequent in spring and to a lesser extent summer than in autumn and winter (ANOVA: d.f. 3, F 27.23, $P < 0.001$) (Fig. 3). PC2 shows that at lower MAP and higher temperature variance (thus more inland), flowering is more common in winter and spring, whereas with higher MAP and lower temperature variance (thus more along the south coast), flowering is significantly more frequent in summer and to a lesser extent autumn (ANOVA: d.f. 3, F 15.7, $P < 0.001$). PC3 reveals a weak tendency for flowering to be more likely in summer in areas with lower seasonality in rainfall (ANOVA: d.f. 3, F 4.041, P = 0.0072). PC4 did not result in a significant result.

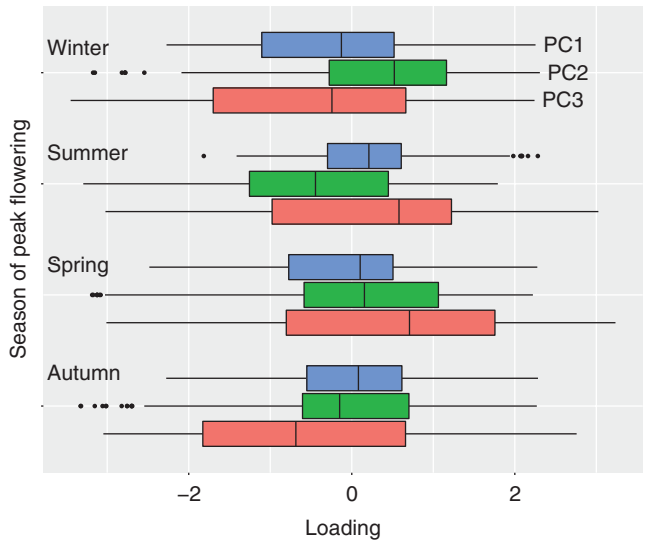

FIG. 3. Restio community peak flowering, simplified to seasons (summer = December, January, February; autumn = March, April, May; winter = June, July, August; spring = September, October, November) plotted against the first three principal component axes, giving ranges, quartiles and medians.

Over the whole year, 27 % of the peak flowering communities are wetland communities. In spring (from August to January), the proportion ranges from 31 to 53 %, and in autumn (February to July) from 5 to 24 %, with June as an outlier with 34 %. The largest proportion of wetland communities flower in November. The differences in proportions of wetland and dryland plots between spring and autumn is significant (Fisher exact test statistic = 0.0117). Thus peak flowering in wetland communities is in spring and summer, from September to January, and proportionally more dryland communities flower in autumn (Fig. 2).

Evolution of flowering times

Restio species have two flowering peaks: in spring (September–October) when a third of all species flower, and in autumn (March–April) when just under a quarter of the species flower. There are some species flowering in every month of the year. The smallest number of species flower in the driest month (January, 40 spp) and the coldest month (June, 50 spp) (Fig. 4). Parsimony mapping of flowering times (Fig. 5) shows that this is highly variable across the tree.

TABLE 1. *Loadings on the principal component axes, and proportion of variance explained*

	PC1	PC2	PC3	PC4
Elevation	**0.599707**	–0.09318	**0.267257**	0.122052
Mean annual temperature	**–0.55246**	0.306828	–0.25541	–0.08573
Temperature variance	0.436294	**0.452104**	–0.17084	**0.329777**
Mean annual precipitation	0.008711	**–0.74965**	0.009553	–0.21074
Maximum rainfall month	–0.34274	–0.23397	0.237428	**0.877927**
Rain seasonality	0.165046	–0.2758	**–0.88187**	0.232016
Proportion of variance explained	0.412	0.26	0.163	0.123
Relationship to geography				
Adjusted R^2 longitude	0.003	0.163	**0.425**	0.001
Adjusted R^2 latitude	**0.173**	**0.179**	0.157	**0.40**
Correlation community mean flowering times				
$R^2_{x\theta}$ community peak flowering time	0.082	0.060	0.027	0.009
P-value	<0.001	<0.001	<0.001	0.136

The two largest loadings for each principal component are in bold. The results of a linear regression of the axes against latitude and longitude (all of which were significant) are reported in the middle block. The lowest block reports the correlation with community peak flowering time

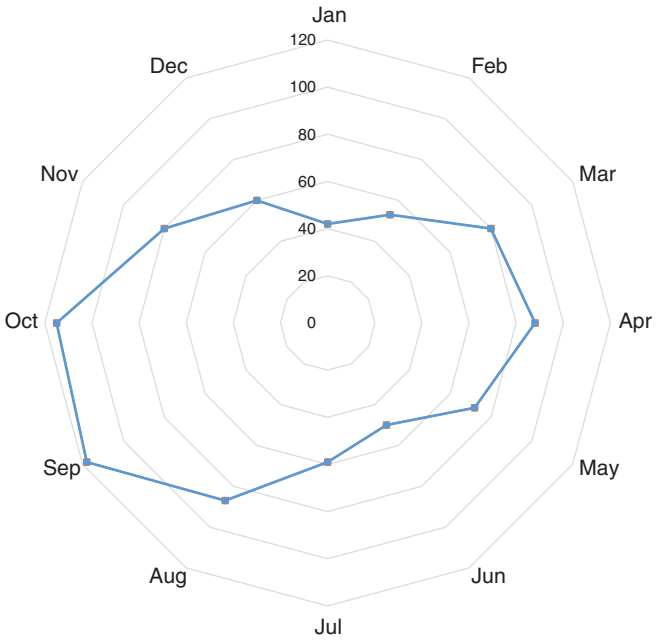

FIG. 4. Number of restio species flowering in each month, based on the full flowering range of each species.

The analysis shows that the restio ancestral mid-range flowering time is late spring–early summer, September–October. Some clades show shifts: for example, to November–December in the *Elegia capensis-stokoei* clade and the *Restio sieberi-elsieae* clade, and to July for the *Thamnochortus* and *Restio* subgen. *Craspidolepis*. Striking, however, are the numerous sister species with autumn/spring shifts in flowering (e.g. *Thamnochortus bachmannii*/*T. punctatus* and *Ceratocaryum argenteum*/*C. pulchrum*). When flowering time is simplified to mid-range flowering time per species and linearized, then neither Blomberg's K nor Pagel's λ are significant (K = 0.1191, $P = 0.1648$; λ = 0.0146, $P = 0.7854$), thus failing to reject the hypothesis of no phylogenetic constraint in the evolution of the median flowering time.

Flowering shift with speciation

Of the 112 sister species pairs on the MCC tree, 59 have (partially) overlapping flowering times (synchronous), and 53 have non-overlapping flowering times (allochronous). In some instances, both sisters flower for only 1 month, and the months are adjacent (thus minimally allochronous), whereas in others, one species flowers in spring and the other in autumn (maximally allochronous). Allochronous sister species, compared with synchronous sister species, are not more likely to be sympatric (thus separate flowering times do not complement geographical isolation). There is no significant difference in the proportions of allopatric to sympatric, compared with allochronous to synchronous, species pairs (Table 2). These proportions remain the same when only sister species pairs with a posterior probability of >0.8 (87), 0.9 (82) and 0.95 (79) are analysed, indicating that the results are robust. There is no significantly larger or smaller climatic or habitat difference between synchronous or allochronous species pairs, but sympatric species pairs have significantly smaller climatic (but not habitat) differences than allopatric species pairs (Table 3).

After removing shift species from the analysis, both λ and K remain marginally non-significant (K = 0.1347, $P = 0.1001$; λ = 0.123, $P = 0.02$), indicating a stronger phylogenetic signal than if shift species are included. Fitting models to the mid-range flowering time per species over the MCC tree (Table 4) shows that for the complete dataset, there is no significant difference between the two best models, λ and Ornstein–Uhlenbeck, but that λ (albeit with a very small value of λ) is significantly better if 'shift species' are excluded. κ is very small for the complete dataset, suggesting that evolution in flowering time appears to be independent of time (e.g. branch length), and so is more associated with the number of speciation events, but is much larger for the dataset without shift species. The δ value of 3 indicates that evolutionary change is concentrated at the tip of the tree, rather than randomly distributed or concentrated near the base.

Flowering shift with environment

The regression analysis of the complete dataset (including MAP, rainfall seasonality, peak rainfall month, MAT, temperature variance, soil moisture and dispersal mode) returned a low R^2 of 5.9 %, with only MAT significant (Table 5). The λ correction is very near zero, consistent with the results above, and indicating little or no phylogenetic conservatism. Repeating the analysis including only species found in well-drained habitats (and so leaving out the edaphic variable) returned the same set of predictor variables (albeit with stronger significance for MAT), but with an R^2 of 10.0 %. Leaving out species with longer flowering times resulted in a further marginal increase in the R^2 to 10.4 %; the temperature variance was retrieved as marginally significant ($P = 0.081$). Repeating the analyses without the 'shift species' retrieves similar results, except that the R^2 values are higher (7.9 % for the full dataset and 14.7 % for only species from well-drained habitats).

DISCUSSION

Community flowering time

Cape restios as a whole flower throughout the year, with a major peak in spring and a minor peak in autumn. This is particularly evident from the community peak flowering times. The low points for both overall numbers of species flowering and community peak flowering are the hottest (January) and the coldest (June) months. This asymmetric bimodal flowering pattern is unusual for the Cape flora, where an analysis of the flowering times of 83 % of the species showed a spring peak, with a gradual tailing off during autumn into winter (Johnson, 1993), but is known for several geophytic groups, such as Amaryllideae (Snijman and Linder, 1996) and orchids (Linder and Kurzweil, 1999). This also differs from the patterns in the Australian Restionaceae, which flower predominantly in late

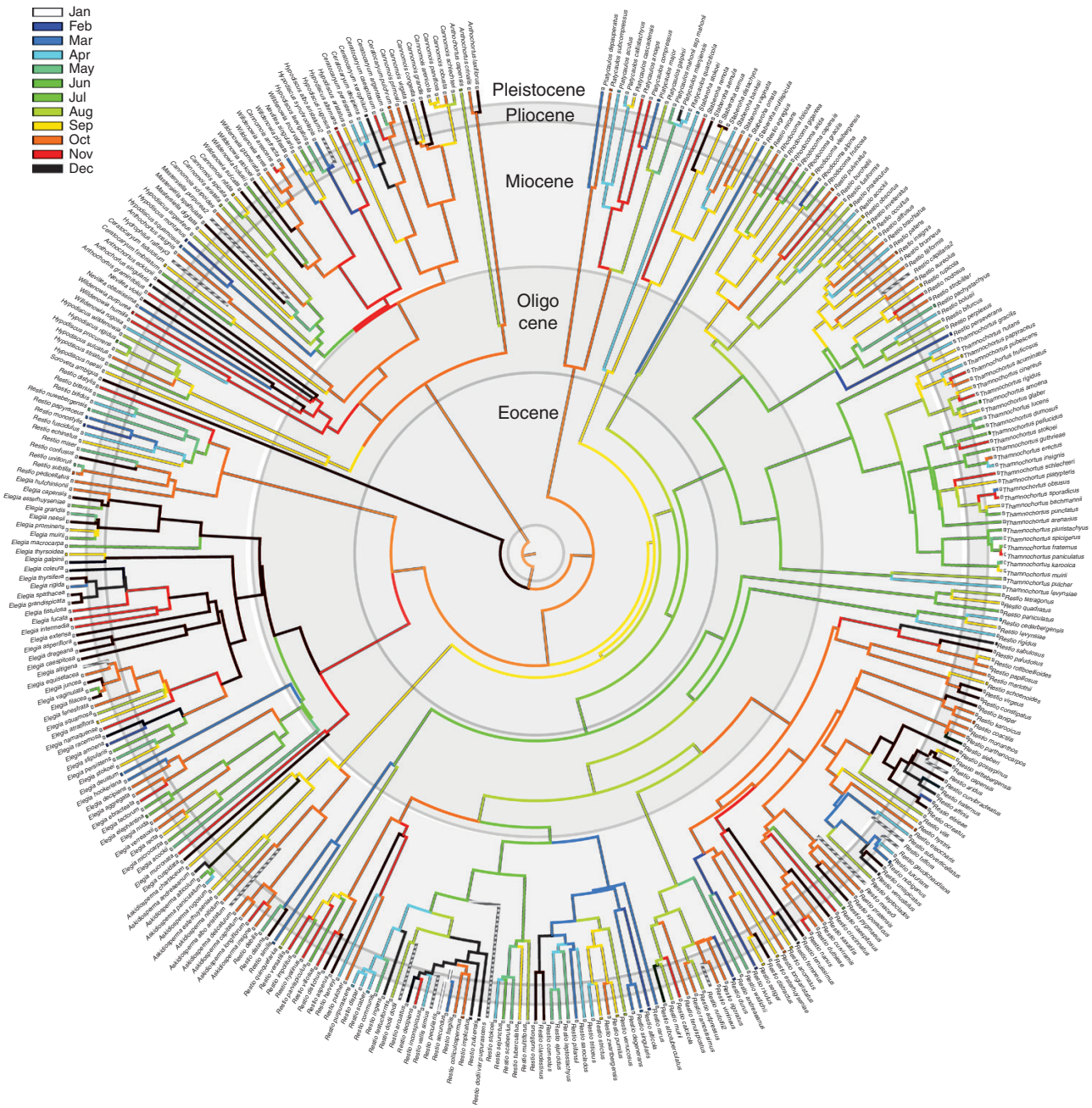

FIG. 5. Phylogeny of Restionaceae showing reconstruction of ancestral flowering times, colour coded by month, over the maximum clade credibility tree, using parsimony optimization with a stepmatrix that weights transitions dependent on the temporal separation between the months. The ancestral flowering time (brown) is optimized to be October; shifts to earlier flowering times are coloured yellow (September) and several shades of green (August to May), whereas red (November), black (December), white (January) and shades of blue (February to April) indicate shifts to later flowering times.

TABLE 2. *Numbers of allochronous and synchronous, as well as allopatric and sympatric restio sister species pairs*

	Allochronous	Synchronous	Totals
Allopatric	25	18	43
Sympatric	28	41	69
Totals	53	59	112

Sympatric species pairs are more often than expected synchronous (Fisher exact test, $P = 0.0075$). Allopatric species pairs are only marginally more often allochronous than sympatric species pairs (Fisher exact test, $P = 0.0821$).

winter and summer (86 %), with only 13 species flowering in late summer and autumn (Meney and Pate, 1999). However, asymmetric bimodal flowering has been demonstrated for the Greek phrygana (Petanidou *et al.*, 1995) and may be common for the Mediterranean flora (Thompson, 2005). As found here for Cape restios, in the Mediterranean there is almost no flowering in mid-winter or mid-summer. This may reflect climatic suitability, with the summers too dry and winters too cold; in spring the soil is still wet from the winter rains but it is warm, whereas in autumn the temperatures and rainfall may be suitable, but the soils are still dry after the summer

TABLE 3. *Comparison of climate and habitat differences between allo- and synchronous, and allo- and sympatric species pairs*

		Allochronous/ allopatric	Synchronous/ aympatric			
Time	Climate*	1.3077	1.3574	$t = 0.429$	d.f. = 109.9	$P = 0.6684$
	Habitat†	0.5283	0.5085	W = 1601		$P = 0.8027$
Geography	Climate*	1.5596	1.1932	$t = -3.360$	d.f. = 101.86	$P = 0.001$
	Habitat†	0.5814	0.4783	W = 1456		$P = 0.8515$

*Climatic differences: the sum of the differences in mean annual precipitation, rainfall seasonality, dry quarter rainfall and minimum elevation, where these have been ranged from 0 to 1

†Habitat differences: the difference in ground water conditions (permanently wet, seasonal seepages, habitats with shallow groundwater, and well-drained habitats) treated as a meristic character.

TABLE 4. *Evolutionary model fitting results for the mean flowering times of the restio species over the maximal clade credibility phylogeny*

	Complete dataset		Excluding sister species allochronous with ancestral state ('shift species')	
Model	AICC	Model parameters	AICC	Model parameters
Lambda	65.22	$\sigma^2 = 0.001$, $\lambda = 0.0146$	30.288	$\sigma^2 = 0.001$, $\lambda = 0.123$
Ornstein–Uhlenbeck	65.45	$\sigma^2 = 0.3796$, $\alpha = 2.7183$	35.36	$\sigma^2 = 0.571$, $\alpha = 0.4367$
Kappa	172.12	$\sigma^2 = 0.0295$, $\kappa = 0.1494$	110.99	$\sigma^2 = 0.0255$, $\kappa = 0.5386$
Delta	238.12	$\sigma^2 = 0.0036$, $\delta = 3$	165.45	$\sigma^2 = 0.003$, $\delta = 3$
Brownian motion	296.03	$\sigma^2 = 0.001$	216.95	$\sigma^2 = 0.01$

TABLE 5. *Phylogenetic regression results for full dataset of restios, using the maximal clade credibility tree and scaled predictor variables*

	Estimate	s.e.	*t*-value	*P*-value
(Intercept)	0.54312863	0.04601768	11.8026	<2.2e-16
MAP	0.00094953	0.02158000	0.0440	0.964931
MeanR	–0.01446237	0.01511771	–0.9567	0.339444
MAT	**–0.06133126**	**0.02062690**	**–2.9734**	**0.003162**
TVar	–0.03030631	0.02397556	–1.2641	0.207104
Edaphic	–0.00164605	0.01514295	–0.1087	0.913506
Dispersal	0.01084496	0.02063324	0.5256	0.599515
Season	0.01103864	0.01606225	0.6872	0.492414

MAP, mean annual precipitation; MeanR, month with most rainfall; MAT, mean annual temperature; TVar, temperature variance; Edaphic, ground water availability; Dispersal, dispersal mode; Season, rainfall seasonality. The significant variable is in bold.

drought. These patterns contrast with the strictly unimodal, summer flowering of the Northern Hemisphere temperate flora. Tropical floras, in contrast, may flower throughout the year. For example, on Barro Colorado island in central Panama, flowering is spread throughout the year, albeit with several peaks, and many species flower for up to 9 months (Croat, 1969). A similar pattern was also documented for the Melastomataceae along the Brazilian Atlantic coast (Brito *et al.*, 2017), where most species flowered at least 6 months a year. Generally, flowering in the Neotropics appears to occur throughout the year, sometimes with a single peak, and sometimes with two flowering peaks (e.g. Morellato and Leitao, 1996; Justiniano and Fredericksen, 2000), and sometimes with minimal flowering during the dry season (e.g. Delampe *et al.*, 1992). Restios are all wind pollinated, and the timing of wind pollination is often linked to other phenological phases, such as leaf deciduousness (Whitehead, 1969). In the evergreen Cape flora, restios are part of the 'canopy vegetation', and so not dependent on leaf phenology.

The largest proportion of wetland Cape restio communities have their peak flowering in spring and summer. This differs from the pattern documented for the Australian Restionaceae (Meney and Pate, 1999), where dryland species flower mostly in late winter and early spring, and wetland species in late spring and summer, consistent with the hypothesis that ground water compensates for the lower summer rainfall. The low proportion of wetland communities of Cape restios flowering in autumn suggests that different factors may be driving community peak flowering in the Cape and Australia. The impact of the seed release time on flowering time is obscure. Restio seed germination in the Cape is mostly at the beginning of winter (June–July, pers. obs.) but, as in the Australian Restionaceae (Meney and Pate, 1999), seed development time is between 2 and 12 months. Furthermore, as seed germination mostly follows fire (thus every 5–40 years), there is likely to be a significant soil residence time. These two factors may reduce the impact of seed germination time on optimal flowering time.

Previous flowering time research in the Cape suggested a geographical pattern, with the species in the west tending to flower in spring, and those to the east tending to flower in autumn (Johnson, 1993). The results obtained here are weakly consistent with this pattern. However, there has been no previous analysis of community peak flowering time in response to habitat or climate in the Cape, and the results obtained here suggest that this geographical pattern may be climatically driven, with restio community peak flowering avoiding extremes (Fig. 2). In hot dry areas (such as Namaqualand, the intermontane valleys or lower mountain slopes), restio flowering tends to be in winter, and in cold, wet areas (such as at higher elevations) in summer.

Evolution of flowering time

Restio flowering time is not phylogenetically constrained and appears to be evolutionarily labile, consistent with the findings of Warren *et al.* (2011) that restios, in contrast to much of the Cape flora, do not retain a signal of late Miocene climate change. The evolution of flowering time in the restios probably results from a combination of shifts with speciation and environmental adaptation. The effect of environmental tracking is weak and explains only 6 % of variation in flowering time. This is largely temperature driven. When tested for only the dryland species, almost 10 % of the variation is explained, but it is not evident why the temperature signal is weakened by wetland species.

Flowering time shifts are often associated with speciation, and almost half of all sister species pairs are allochronic (54 of the 112), whereas allopatry is found in only 44 sister species pairs. However, there is no direct evidence that allochrony associated with speciation is more frequent than expected, because a sensible null model remains elusive. However, there are several indications that the association between flowering time shift and speciation is higher than expected. Firsty, if flowering time is inherited, then sister species should have synchronous flowering, and this is not consistent with almost half the sister species pairs being allochronic. Secondly, the lack of phylogenetic signal in mid-range flowering time suggests evolutionarily labile flowering time, and this is corroborated by the high δ and low κ, suggesting that most evolutionary change occurs at the tips of the phylogeny. This association between speciation and flowering time change is stronger than reported by van der Niet and Johnson (2009), and their observation that this was higher than for other Cape clades suggests that shifts in flowering time may be more commonly associated with speciation in restios than in other Cape clades. For wind-pollinated taxa, flowering time shifts are one of the few ways to achieve reproductive isolation. There is, however, no link between geographical isolation and flowering time shifts; almost equal numbers of allochronic sister pairs are allo- or sympatric and, for synchronous sister pairs, 41 are sympatric and only 18 allopatric. Consequently, there is no support for the hypothesis that they function as alternative speciation modes.

My results corroborate the suggestion by van der Niet and Johnson (2009) that allochronic reproductive isolation could be an important speciation mechanism in restios, and allochrony appears to be at least as important as allopatry. However, post-speciation range expansion may erase the signal of allopatry (Barraclough and Vogler, 2000; Losos and Glor, 2003). If the range sizes are ecologically controlled, then Quaternary climate fluctuations are likely to have resulted in range changes, which may have obliterated the signal of allopatric speciation. In contrast, flowering time is likely to be under stabilizing selection (especially in a wind-pollinated dioecious clade), as early or late flowering outlier individuals will leave no offspring, and this could strengthen the signal of allochrony. Although some species are clonal, at least 116 of the 350 species are killed by fire and re-establish from seed (this number is most probably a strong underestimate; the real value could be closer to 240 species) (Linder, 2001), underlining the importance of effective seed production. These two processes may enhance the signal of allochronic speciation through time.

The lack of more frequent habitat shifts between allochronic and synchronic sister species argues against flowering time tracking habitat changes. This is consistent with the allochronic speciation model (Taylor and Friesen, 2017), rather than straightforward ecological speciation in response to habitat changes, aided by assortative mating. In the actual speciation process, flowering time shifts could be accentuated post-divergence in sympatric species (e.g. *Viburnum*; Spriggs *et al.*, 2019), and hence flowering time shifts may result in assortative mating within the segregating sister species, and this process can be very powerful if there is large flowering asynchrony (Weis *et al.*, 2014). This could drive sympatric speciation (Coyne and Orr, 2004; van der Niet and Johnson, 2009). Devaux and Lande (2008) used a modelling approach to show that allochronic speciation is possible without disruptive ecological selection, especially where (*a*) individual flowering times are short within a long potential flowering season; (*b*) there is no pollinator limitation; and (*c*) plants are self-incompatable; (*d*) have high mutation variation; and (*e*) have multigenic flowering time inheritance. Restios fit the first three criteria well: the Cape flowering season lasts the whole year albeit with two peak seasons; restios, as wind-pollinated plants, have no pollinator limitation in the windy Cape, and as dioecious plants show complete self-incompatability. However, we have no information on the mutation rate or the nature of flowering time inheritance. A possible interaction between occasional flowering time shifts and allochronic speciation perpetuating these shifts is consistent with phylogenetic lability in flowering time, which is not strongly linked to environmental variables. Consequently, it seems probable that allochronic speciation is frequent and widespread in restios. Such allochronic speciation could be regarded as non-adaptive. A non-adaptive radiation may fail to show density-dependent slowdown, and indeed this was demonstrated for the restios (Bouchenak-Khelladi and Linder, 2017).

Numerous speciation mechanisms have been proposed, with varying degrees of support, for the Cape flora. Allopatric speciation may be a common geographical mode (Schnitzler *et al.*, 2011; Verboom *et al.*, 2015). Johnson (1996) made a coherent argument, subsequently supported by further evidence (van der Niet and Johnson, 2009), for the importance of pollinator shifts in establishing reproductive isolation. Linder (1985) argued that steep selective gradients and limited short-distance gene flow facilitated speciation in the Cape region. Here I argue that allochronic speciation may also be an important mechanism in the Cape flora, particularly for wind-pollinated species. This may account for the exceptional species richness of Cape wind-pollinated clades (Koutnik, 1987); it seems that this could be a consequence of the environmental conditions in the Cape favouring allochronic speciation.

Flowering times within Cape restio communities appear to be dominated by environmental filtering, and community peak flowering time correlates, albeit weakly, with temperature and water availability, both as rainfall and as ground water. This contrasts with the evolution of flowering time, which provides the raw material for assembling communities, and which appears to be in significant part a by-product of allochronic speciation. There is a weak signal of flowering time adaptation to temperature, but this is not correlated with

allochronic sister species. Flowering time evolution is consequently driven by biotic rather than abiotic factors. This unexpectedly high frequency of allochronic speciation may be a side effect of wind pollination and lack of dependence on pollinator activity times, and of the Cape climate system with its particularly weak seasonality. In the restios, the processes generating flowering time diversity among species appear to be different from those influencing the community peak flowering time. Most probably these different processes operate on different time scales, with flowering time evolution associated with speciation probably on a million year time scale, whereas community assembly could operate on a much shorter decadal or century time scale. Perhaps more important is that the contexts are totally different: flowering time evolution occurs in a phylogenetic framework, whereas community peak flowering occurs in an ecological–spatial framework. Linking these two frameworks to account for both ecological and phylogenetic results presents an interesting challenge.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: species flowering time, traits and environment. Table S2: plot flowering times and plot attributes. Table S3: stepmatrix used to map flowering time over phylogeny in Mesquite. Table S4: sister species dataset.

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