

Temporal shifts in reproductive phenology of cycads: a comparative study in *Ceratozamia*

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Abstract

The reproductive phenology in plants consists of successive life cycle phases leading to reproductive success. In seed plants, cycads and other dioecious groups have complex reproductive systems, where individuals require synchronizations among two sexes and populations of the same species. Here, we analyzed phenology between populations of three geographically close species in the cycad genus *Ceratozamia* Brongn. We described the lifespan of pollen and ovulate strobili and their morphological changes throughout ontogeny and evaluated synchrony among reproductive events, focusing on the timing and abundance of the receptivity and open pollen phases. Our results showed that the reproductive timing in the three species was highly synchronous, and that the overlap between the receptivity and open pollen phases could point to gene flow among populations of different species. We identified a correlation between the reproductive patterns and the temperature and precipitation regimes. Pollen and ovulate strobili were produced during the rainy season, whereas the period of synchrony between receptivity and pollen occurred during the dry season. The seed release occurred during the rainy season, which could contribute to keep the seed moist and prevent the embryo from drying out. Finally, we highlight the utility of studies in wild populations for the ecological and evolutionary understanding of phenological patterns in cycads.

Key words: dioecy, evolution of phenological patterns, gymnosperm ecology, phenophase, *Zamiaceae*

Résumé

La phénologie de la reproduction chez les plantes consiste en phases successives du cycle de vie menant au succès reproductif. Chez les plantes à graines, les cycades et d'autres groupes dioïques possèdent des systèmes reproductifs complexes, où les individus ont besoin d'une synchronisation entre les deux sexes et les populations de la même espèce. Les auteurs ont analysé ici la phénologie entre les populations de trois espèces géographiquement proches du genre *Ceratozamia* Brongn. Ils ont décrit la durée de vie du pollen et des strobiles ovulifères et leurs changements morphologiques tout au long de l'ontogenèse et évalué la synchronisation entre les événements reproductifs, en se concentrant sur la coordination et l'abondance des phases de réceptivité et polliniques ouvertes. Leurs résultats ont montré que la coordination de la reproduction des trois espèces était hautement synchrone, et que le chevauchement entre les phases de réceptivité et polliniques ouvertes pourrait indiquer un flux génétique entre les populations de différentes espèces. Ils ont identifié une corrélation entre les patrons de reproduction et les régimes de température et de précipitations. Le pollen et les strobiles ovulifères étaient produits pendant la saison des pluies, tandis que la période de synchronisation entre la réceptivité et le pollen avait lieu pendant la saison sèche. La libération des graines avait lieu pendant la saison des pluies, ce qui pourrait contribuer à maintenir la graine humide et à empêcher l'embryon de se dessécher. Enfin, les auteurs soulignent l'utilité des études sur les populations sauvages pour la compréhension écologique et évolutive des modèles phénologiques chez les cycades. [Traduit par la Rédaction]

Mots-clés : dioécie, évolution des modèles phénologiques, écologie des gymnospermes, phénophase, *Zamiaceae*

Introduction

The temporal patterns of biological events in plants, such as leaf production and timing of flowering or coning, con-

stantly interact with the prevailing environmental dynamics of an area (Cleland et al. 2006; Arcanjo-Bruno et al. 2019). These plant life-cycle events are often linked with climatic

variables (Menzel 2002). Recently, phenological studies have increased in number because climate change could cause mismatches in the interactions between plants and their ecological role in communities (Walther et al. 2002; Gordo and Sanz 2010; Numata et al. 2022). The variable responsiveness of plants to local conditions could even have considerable consequences within reproductive systems, and negatively impact species survival (Walther et al. 2002; Forrest and Miller-Rushing 2010; Gordo and Sanz 2010; Stucky et al. 2018). The degree of response to environmental seasonality within these types of events can vary in magnitude of influence, both across different developmental events — or phenophases — and between species (Martins et al. 2021).

Because seasonal droughts can be long in arid and semi-arid regions, the close relationship between environmental patterns and reproductive events is more conspicuous, particularly in relation to temperature and precipitation regimes, which could initiate the phenophases (Renzi et al. 2019; Sakai and Kitajima 2019). In tropical forests, seasonal timing and factors that may explain phenological changes have been highly diverse or have shown large variation (Sakai and Kitajima 2019). Associations of phenological patterns with climate variables at the community level have been the focus of several phenological research projects (Stradic et al. 2018). This large-scale approach has allowed for the detection of macrogeographic patterns. Although the phenology in closely related species tends to be similar, the approaches that are restricted to species within the same taxonomic group allow the visualization of the heterogeneity of phenological patterns, as well as the discovery of the drivers of phenological transitions and how these are synchronized between related species (Davies et al. 2013; Gerst et al. 2017). Phenological patterns provide crucial data to understand plant reproduction, because seasonal timing in phenology is directly linked to fertilization (Inouye et al. 2003). Shifts in reproductive timing can act as reproductive isolation mechanisms or as drivers of speciation (Morellato et al. 2010; Christie and Strauss 2018). Conversely, shifts that lead to congruent phenologies can reduce reproductive barriers and lead to hybridizations (Miller-Rushing et al. 2010; Clugston et al. 2016). In line with this, phenology can also provide evidence to infer evolutionary adaptations and phylogenetic relationships (Clugston et al. 2016). Phenological studies in dioecious species — particularly those that require external vectors for successful pollination — could have outstanding research value due to their susceptibility to environmental changes (Miller-Rushing et al. 2010; Clugston et al. 2016). These species require spatial and seasonal reproductive synchronization both between plants that produce male and female gametes and between species (Lazcano-Lara and Ackerman 2018). However, most plant phenological research has focused on monoecious species (Escobedo-Sarti and Mondragón 2016; Martins et al. 2021), leaving dioecious species with long life cycles relatively unattended.

Gymnosperms in the order Cycadales are a group of tropical to subtropical dioecious taxa that are characterized by cross-pollination. Specialized mutualistic interaction between cycads and their pollinator insects is essential for their mutual survival (Terry et al. 2014). In paleobotanical terms,

cycads are an ancient group which dates back to the Upper Paleozoic (265–290 million years ago), but their relatively higher diversity in the past contrasts with their current status as one of the most threatened plant groups with a significant extinction risk (Nicolalde-Morejón et al. 2014). Thus, efforts to characterize their phenological cycles and the traits that characterize them become more pressing from a plant conservation biology viewpoint.

Recently, cycad phenology has been approached from phylogenetic and taxonomic perspectives (Clugston et al. 2016; Martínez-Domínguez et al. 2018a; Segalla et al. 2021). In Mexico, these types of studies are promising due to the occurrence of three genera — namely, *Zamia* L., *Dioon* Lindl., and *Ceratozamia* Brongn. The latter genus is the most diverse in this area and its species are pollinated by insects of the Erotylidae family (Nicolalde-Morejón et al. 2014; Tang et al. 2018). *Ceratozamia* species have long reproductive cycles of approximately one year, but phenological phases have been described in only one species, *Ceratozamia tenuis* (Dyer) D.W.Stev. & Vovides (Martínez-Domínguez et al. 2018a).

Generally, the traits evaluated in phenological studies are duration, onset, and termination of phenophases (Renzi et al. 2019). Peaks in these parameters are crucial to show the tendency that related species might have to exhibit either the same phenological profile or overlaps in their patterns (Renzi et al. 2019). The research presented here was designed to fully document and characterize the reproductive cycles, including phases in the ontogenetic development of reproductive structures in three species of *Ceratozamia* that inhabit in the central region of Veracruz State (Mexico): (i) *Ceratozamia morettii* Vázq.Torres & Vovides; (ii) *Ceratozamia brevifrons* Miq.; and (iii) *Ceratozamia delucana* Vázq.Torres, A.Moretti & Carv-Hern. Additionally, we explored the relationships among phenophases and climatic variables in these species. Thus, the aims of this study were as follows: (i) to characterize the phenophases in each species; (ii) to examine the onset, termination, duration, and peaks of phenophases, as well as their relationship with climatic variables; and (iii) to compare the degree of synchrony among species.

Materials and methods

Species and sites of study

Ceratozamia morettii and *C. brevifrons* each have a narrow distribution. The former is a little-known cycad that occurs in the cloud forest, on karstic rocks and cliffs between 1200 and 1850 m of elevation, whereas *C. brevifrons* inhabits karstic rocks in the oak forest and the transition zone between oak forest and cloud forest between 500 and 1350 m. In contrast, *C. delucana* is a species with wide distribution from the northern central region of Veracruz down to the Puebla state. It grows in the cloud forest, between 500 and 1650 m of elevation. Morphologically, these three species bear close similarities, but they can be distinguished by certain vegetative characters, such as type and number of prickles and insertion of leaflets on the rachis. In particular, *C. delucana* is a polymorphic species similar in both vegetative and reproductive morphology to *C. morettii* (cf. Martínez-Domínguez et al. 2018b).

The three species occur in the cloud forest region, informally known as “mountain range of Puebla-Chiconquiaco”, which is part of the Sierra Madre Oriental (CONABIO 2008). The study was carried out in two populations of each species occurring along this area, within the distribution range of the three species (Fig. 1). The Sierra Madre Oriental is a mountain range located in Eastern Mexico, spanning from the central coastal plain of the Gulf of Mexico toward the central high plateau of Mexico (Eguiluz de Antuñano et al. 2000).

Phenological observations

Observations for reproductive phenological events in all wild populations were carried out from August 2016 to August 2021. A directed sampling scheme was conducted to register each reproductive individual and the ontogenetic stage of their corresponding reproductive structures. Because the study area has steep slopes, the most accessible individuals were studied to guarantee covering the largest possible area containing plants in each of the populations. The populations of *C. morettii* were difficult to monitor because the plants occur in steep and exposed rocky walls. Thus, the number of adult individuals observed in both populations was lower than in populations of the remaining two species included in the study. These populations were visited monthly, when the stage of the reproductive structures (pollen and ovulate strobili) indicated the start of the phenophases associated with pollination and fertilization (i.e., receptivity and open pollen). Sometimes, the individual plants were visited more than once a month. We recorded the phenophases of each individual and the number of reproductive structures at the population level (Table 1). These phenophases were described for each species, following previous descriptions by Martínez-Domínguez et al. (2018a).

Statistical analysis

Circular statistics was applied for observations of receptivity and open pollen using the ORIANA software (Kovach 1994). Months of the year were converted to angles at 30° intervals of the circumference from January to December (Morellato et al. 2010). We calculated the mean angle (μ), which indicates the central tendency of data, the mean vector (r), and Rayleigh's test (z) to evaluate deviations (Zar 2010). We tested data for normality and homoscedasticity tests. We analyzed the correlation among phenological and temperature and precipitation parameters using the non-parametric Spearman correlation. The monthly temperature and precipitation data were obtained directly from the nearest meteorological station of the National Meteorological System of Mexico for the species of interest (Misantla, for populations of *C. brevifrons*; Atzalan, for *C. delucana*; and Naolinco, for *C. morettii*). Because we had incomplete data from the meteorological stations, analyses were carried out using the average of temperatures and precipitation for 2016–2018. For *C. delucana*, temperature data were not incorporated because the meteorological station only registered precipitation data during this period. This set of analyses was performed using the R software (R Core Team 2013).

Results

During this time period, we registered 43 polliniferous plants and 21 ovuliferous plants for *C. morettii*. A total of 24 ovuliferous plants and 53 polliniferous plants were registered for *C. brevifrons*, and 67 ovuliferous plants and 102 polliniferous plants for *C. delucana*. Ovuliferous plants produced only one strobilus per apex, while polliniferous plants produced up to two strobili per apex. The strobili of both polliniferous and ovuliferous plants emerged from the shoot apex surrounded by cataphylls. The ovuliferous plants did not produce a strobilus each year, while most of the polliniferous plants did produce a strobilus each year.

Morphological description and lifespan

Pollen and ovulate strobili pass through four stages of the development over their lifespan. In pollen strobili, the first phase was the emergence (EP) stage, which began in August for all species and lasted between three and four months for each pollen strobilus (Fig. 2A). In *C. morettii*, some pollen strobili were recorded earlier in July. At the population level, these structures emerged continuously during the next three months (September, October, and November). This phenophase was characterized by tightly packed microsporophylls, which were yellowish to greenish with orange trichomes. The closed pollen (CP) stage lasted one month and, in a few individuals, up to two months. It was characterized by the complete development of microsporophylls and the pre-elongation of the central axis (Figs. 3B and 4B). Microsporophylls exhibited the same color that would have until maturity. Pollen strobili were greenish to yellowish green with black trichomes during CP in the three species. *Ceratozamia morettii* had abundant trichomes on the distal face of microsporophylls, in contrast to the other two species. The open pollen (OP) phase occurred during the elongation of central axis and dehiscence of the microsporangia. During OP, the microsporangia were visible following the separation of the microsporophylls, and finally dehiscent synangium units released the pollen (Fig. 2C). The OP lasted three to four weeks in each individual plant (Fig. 4C). At the population level, OP occurred from February to June in *C. brevifrons*, from December to June in *C. morettii*, and from January to June in *C. delucana* (Fig. 5). The highest number of OP strobili was recorded from January to March, for *C. brevifrons* and *C. delucana*, and in January for *C. morettii* (Fig. 5D–F). The number of individual plants in OP progressively decreased from April onward. The final phase of lifespan was senescence (SP), in which the apex of pollen strobilus became curved and microsporophylls turned brown as the strobilus structure dried up (Figs. 2D, 3C, and 4D). The SP phase lasted one to two weeks.

The emergence of ovulate strobili (EO) at the population level for all species started during August and finished in September (Figs. 5A–C). This phenophase began with the emergence of ovulate strobili from the shoot apex and lasted until megasporophylls were distinct enough from each other and the peduncle (sterile part of strobilus) was visible (Figs. 2E and 3D). The ovulate strobili were yellowish with orange trichomes, and throughout ontogeny they turned green to dark green with blackish trichomes. On average, the receptivity

Fig. 1. Distribution map of *Ceratozamia brevifrons*, *Ceratozamia delucana*, and *Ceratozamia morettii*. The localities where the phenological monitoring was carried out are represented with large symbols. Map source data were collected from the “Geoportal of CONABIO”. The map was created in ArcMap 10.2 by L. Martínez-Domínguez. [Colour online]

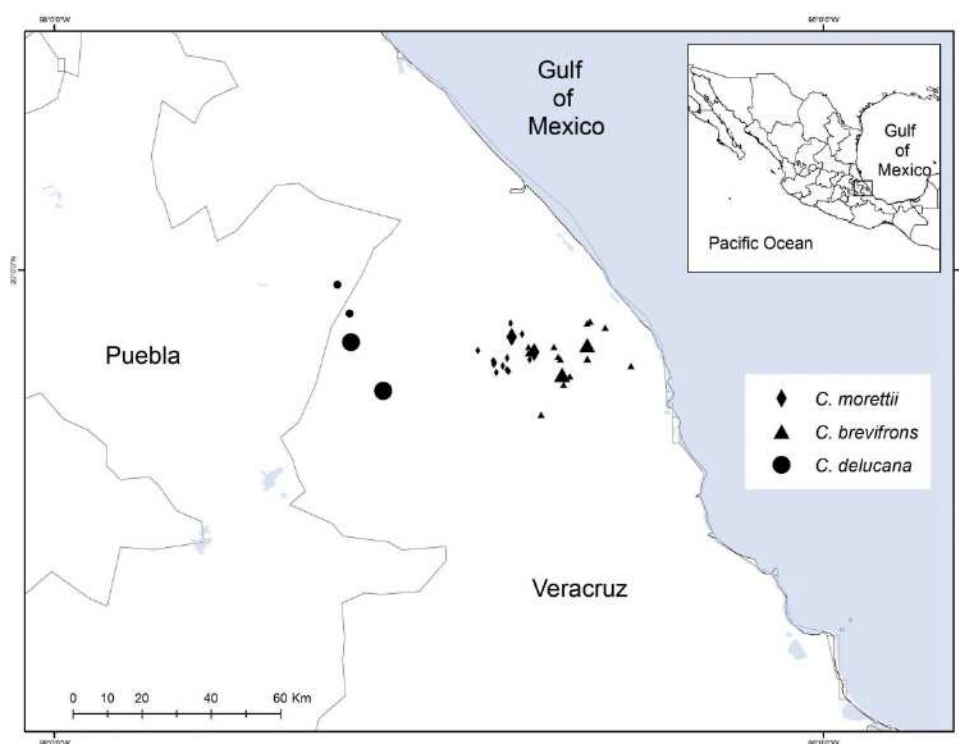


Table 1. Summary of the main descriptive variables in circular statistical analysis for the three *Ceratozamia* species.

Variables	<i>C. brevifrons</i>		<i>C. delucana</i>		<i>C. morettii</i>	
	Receptivity	Open pollen	Receptivity	Open pollen	Receptivity	Open pollen
Mean vector ($^{\circ}$)	56.168	84.653	44.09	56.123	9.418	29.313
Length of mean vector (r)	0.979	0.83	0.951	0.843	0.99	0.78
Median angle, μ ($^{\circ}$)	45	75	45	45	15	15
Rayleigh test, z	23.01	36.52	60.63	72.42	20.59	26.15

(R) phase lasted three weeks. This phase involved the opening and closing of cracks between megasporophylls, and included three events: (i) pre-receptive, in which the base of megasporophylls changed from green to reddish color (8–10 days long); (ii) open-receptivity, in which a reddish color intensified at the base of the megasporophylls, as well as a separation between them (5–10 days long); and (iii) post-receptivity, when the cracks were closed and megasporophylls gradually turned green to yellowish green or light-green at the base (10–15 days long). *Ceratozamia brevifrons* was characterized by yellowish to greenish-yellow megasporophylls during all developmental stages after receptivity. The late ovulate (LO) was the longest phase recorded in which the ovules increased in size and the sarcotesta color became visible. This phase lasted five to six months in each individual plant. In LO, the pollinated ovules began their development but the distal color of megasporophylls remained unchanged. Finally, the disintegration (DO) phase was characterized by the release of megasporophylls from the central axis, and the initiation of sarcotesta degradation. During this

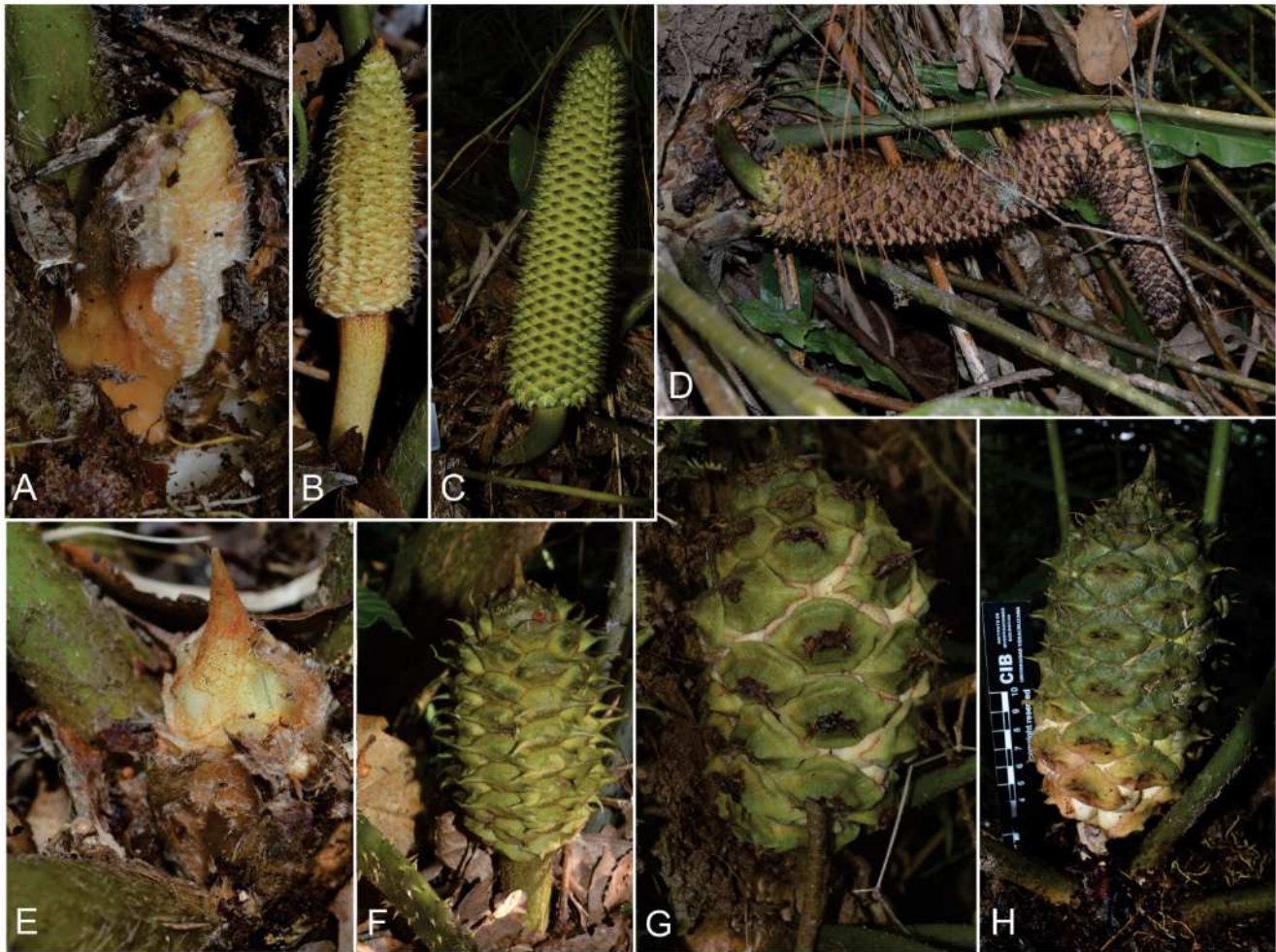
phase, megasporophylls in all species were brownish green. In all species, DO occurred from August to October; however, most plants released their seed in September.

Synchrony of phenological patterns

In general, we observed synchrony between the two populations monitored for each of the species studied. However, polliniferous plants finished the season asynchronously in *C. morettii* and *C. brevifrons* populations. It was not possible to obtain sufficient data for a more detailed comparison between populations of the same species because some populations were small (i.e., composed of approximately 50 individuals, with few of them in reproductive stages) and due to the disparity of reproductive events between years. Monitoring showed that, after a given year with several reproductive individuals in a population, the next yearly period had no ovuliferous strobili and few pollen strobili.

The central tendency of mean angles in phenophases showed that observations were concentrated in the same direction (Table 1). Phenograms showed partial synchroniza-

Fig. 2. Reproductive phenology of *Ceratozamia morettii*. Phenophases for polliniferous plants: (A) emergence (August), (B) emergence (fully emerged, 30 days from beginning emergence), (C) closed pollen (March), (D) senescence (May). Phenophases for ovuliferous plants: (E) emergence (August), (F) pre-receptivity (10 days from beginning receptivity phase — December), (G) late ovulate (May), (H) disintegration (August). [Colour online]



tion in the receptivity and open pollen phases within the same species (Fig. 5). We observed an asynchrony between R and OP during most of the months in the reproductive cycle. This is because at the population level, the R phase occurred for two or up to three months, whereas OP occurred continuously for three to four months after receptivity. Thus, asynchrony involves extra time for pollen shedding. The peaks between these phenophases overlapped within each species. At the population level for all species, the R phase was shorter than OP. In addition, these phenophases showed an overlap between all species, including the general pattern of beginning and end of these phenophases (Fig. 5). *Ceratozamia brevifrons* and *C. delucana* matched in peaks for the R phase, which occurs during February. The peak for the OP phase in *C. brevifrons* occurred in March, while in *C. delucana* occurred in February. In *C. morettii*, the peaks of the OP and R phases matched with each other during January (Fig. 5). The OP phase progressively decreased throughout the year for all species: in *C. brevifrons*, it started in January and lasted until June; in *C. morettii*, it went from December until May;

and in *C. delucana* it started in January and lasted until June. In particular, *C. morettii* was the species where the OP and R phenophases began earlier, during December.

Influence of climate on phenology

In general, the R and OP phases turned out to be correlated with the climatic variables evaluated (Figs. 6–8). There was low negative correlation between the precipitation and R phase, with the exception of *C. delucana* (Table 2). The R phase occurred during the mid-dry season for all species. This phenophase was correlated with low monthly precipitation, but the temperature displayed an irregular pattern (Figs. 6A, 7A, and 8). Despite the similarity in the precipitation regime for the three species, lower rainfall was observed in *C. brevifrons* (Fig. 6). In *C. delucana*, the highest precipitation occurred between September and October (Fig. 8), whereas in *C. morettii*, a longer period of rainfall was recorded beginning in August (Fig. 7). The monthly temperature was lower during the period of receptivity. The peaks for all species occurred

Fig. 3. Reproductive phenology of *Ceratozamia brevifrons*. Phenophases for polliniferous plants: (A) emergence (fully emerged — December), (B) closed pollen (February), (C) senescence (March). Phenophases for ovuliferous plants: (D) emergence (fully emerged, six months from beginning emergence — February), (E) receptivity (March), (F) late ovulate (July), (G) disintegration (October). [Colour online]



within this general pattern, without a clear correlation with the beginning or ending of dry and cold seasons.

The OP phase was more consistently related with the temperature (Table 2). At higher temperatures, the number of pollen strobili decreased (Figs. 6 and 7). The peaks occurred at relatively low temperatures. Precipitation did not increase or decrease consistently between consecutive months. However, precipitation values were the highest during the months of the year when we did not observe pollen strobili (from July to October).

The timing of the E phase, both in pollen and ovulate strobili, occurred during the rainy and warm season. The development of pollen strobili did not show a clear correspondence with seasonality, whereas the development of ovulate strobili occurred during the dry season and the beginning of the warm season. The DO phase occurred at the beginning of the rainy season.

Discussion

Reproductive phenology is a critical part of plant life cycles, particularly with regards to reproductive success (Inouye et

al. 2003; Tang et al. 2016). Because cycads are long-lived and have reproductive structures with long-lifespan, their phenology has been little explored (Stevenson 1981; Clark and Clark 1987; Tang 1990; Ornduff 1991). Recently, phenological data have been used for describing lifespans, analyzing patterns from a phylogenetic context, and synchrony among events (Lopez-Gallego and O'Neil 2010; Martínez-Domínguez et al. 2018a, 2021; Segalla et al. 2021). Living collections and herbarium specimens have been used as a resource for the systematic study of phenology (Calonje et al. 2011; Griffith et al. 2012; Clugston et al. 2016). However, phenological monitoring from wild populations provides complementary relevant data for exploring the biological drivers of phenological transitions.

Phenological studies in cycads provide data for taxonomy, ecology, conservation, and could improve our understanding of evolutionary processes responsible for the current diversity (Griffith et al. 2012). Characters of reproductive structures have been useful to identify species in genera such as *Zamia* and *Ceratozamia* (Nicolalde-Morejón et al. 2009; Martínez-Domínguez et al. 2018b, 2021; Calonje et al. 2019). In particular, the description of ontogenetic stages for ovu-

Fig. 4. Reproductive phenology of *Ceratozamia delucana*. Phenophases for polliniferous plants: (A) emergence (fully emerged — December), (B) closed pollen (March), (C) open pollen (March), (D) open pollen and senescence in the same plant. Phenophases for ovuliferous plants: (E) pre-receptivity (March), (F) post-receptivity (30 days from beginning receptivity phase — March), (G) late ovulate (August). [Colour online]



late and pollen strobili are relevant to detect the stage in which to evaluate the morphological characters that characterize a species (Martínez-Domínguez et al. 2018a). Here, we found that the shape and sporophyll color in pollen and ovulate strobili remained the same during most ontogenetic stages in the three species of *Ceratozamia* (Figs. 2–4). The corresponding character states can be assessed during CP and OP phases in pollen strobili and LO in ovulate strobili.

The reproductive cycle in cycads has two essential phases for reproduction: R and OP, for ovuliferous and polliniferous plants, respectively. These phases must occur simultaneously because pollination is dependent on interactions with insects, which carry pollen from pollen-bearing plants in the OP phase to ovulate strobili that are in the R phase (Stevenson et al. 1998; Terry et al. 2005). Our results showed a slight time difference between these two phases (Fig. 5). At the population level, plants in the R phase were synchronic, whereas plants in the OP phase were partially asynchronous (Fig. 5). Although the number of pollen strobili decreases during the year, some individuals continue to complete their ontogenetic development until reaching the OP phase. This disparity has been described in other cycad species (Martínez-Domínguez et al. 2018a; Segalla et al. 2021), which could be

related to dynamics involving insect pollinators. We have observed individuals of *Pharaxonotha tenuis* (Santiago-Jiménez, 2019) in the last polliniferous strobili of a population in July, and up to four generations of insect pollinators during a year in populations of *C. tenuis*. However, it has not been reported if the insects go into diapause until the next reproductive period as other cycad species such as *Zamia furfuracea* L.f. (Norstog and Fawcett 1989; Norstog et al. 1986). It is not even known if and when the insects stop looking for other pollen strobili, or what are the factors that promote their diapause.

Our results also demonstrated that the phenological patterns of *C. brevifrons*, *C. delucana*, and *C. morettii* are similar to each other, with the latter species displaying a slight disparity (Fig. 5). The R phase started first in *C. morettii*; however, all three taxa do have some plants in the R phase at the same time. This would allow for cross-pollination between all three species; thus, making the production of hybrids possible because these species have similar reproductive peaks (R and OP phases). This temporal overlap could allow some gene flow among populations between these three species. Additionally, *C. morettii* and *C. brevifrons* occur in adjacent areas, and in some localities, they have an interpopulation distance of only 1–1.5 km (Fig. 1) increasing the prob-

Fig. 5. Circular histograms of reproductive phenological events. Phenophases of *Ceratozamia brevifrons*: (A) receptivity and (D) open pollen. Phenophases of *Ceratozamia morettii*: (B) receptivity and (E) open pollen. Phenophases of *Ceratozamia delucana*: (C) receptivity and (F) open pollen. [Colour online]

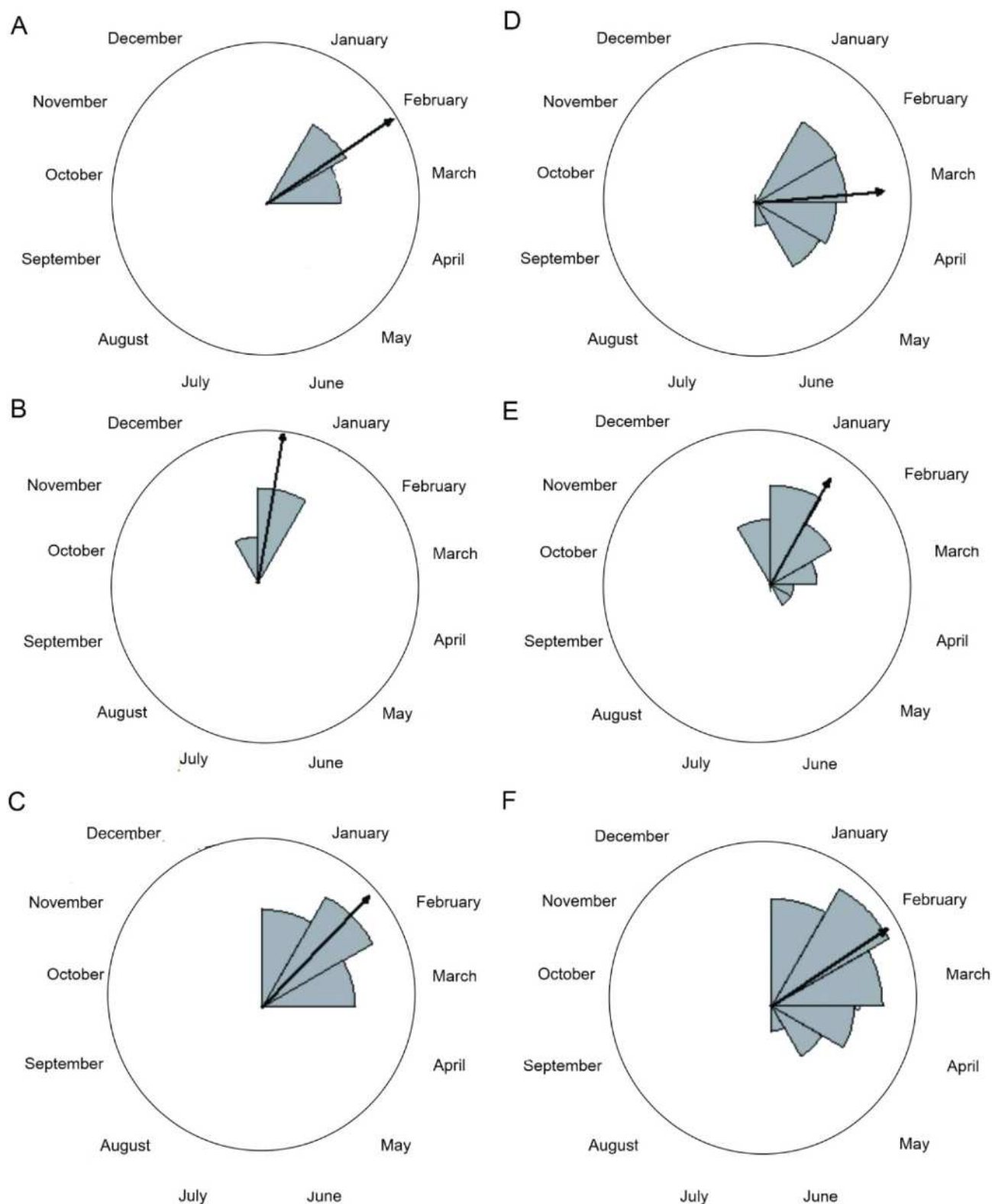


Fig. 6. Average monthly climate for receptivity and open pollen phenophases in *Ceratozamia brevifrons*: (A) precipitation and (B) temperature. [Colour online]

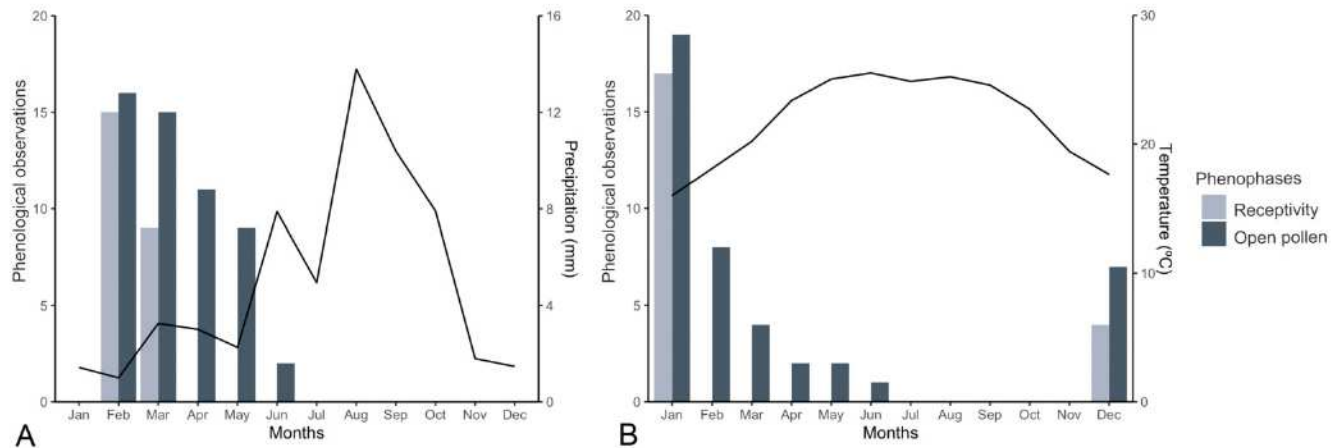


Fig. 7. Average monthly climate for receptivity and open pollen phenophases in *Ceratozamia morettii*: (A) precipitation and (B) temperature. [Colour online]

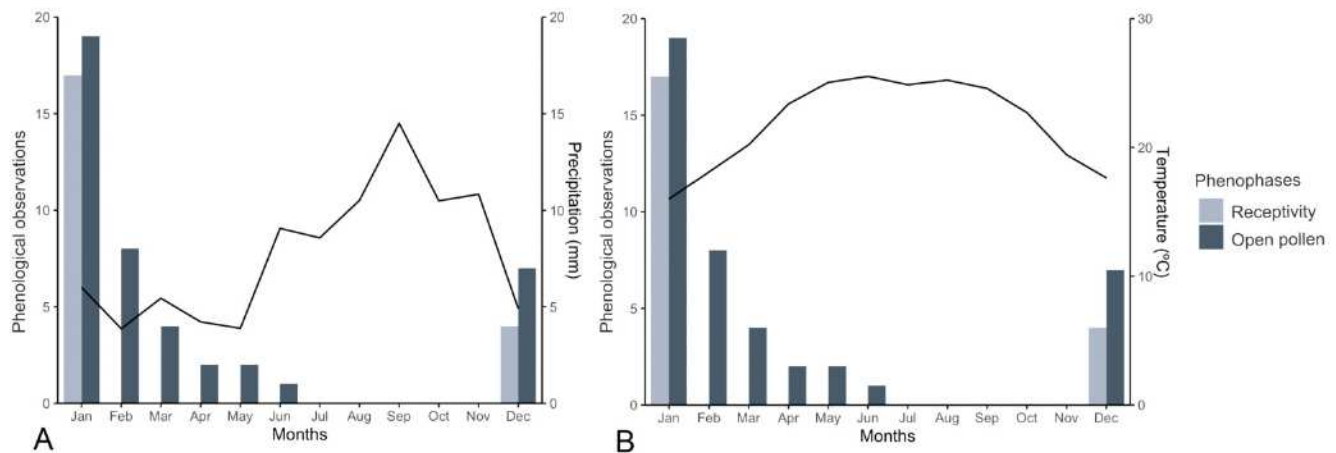


Fig. 8. Average of precipitation for receptivity and open pollen phenophases in *Ceratozamia delucana*. [Colour online]

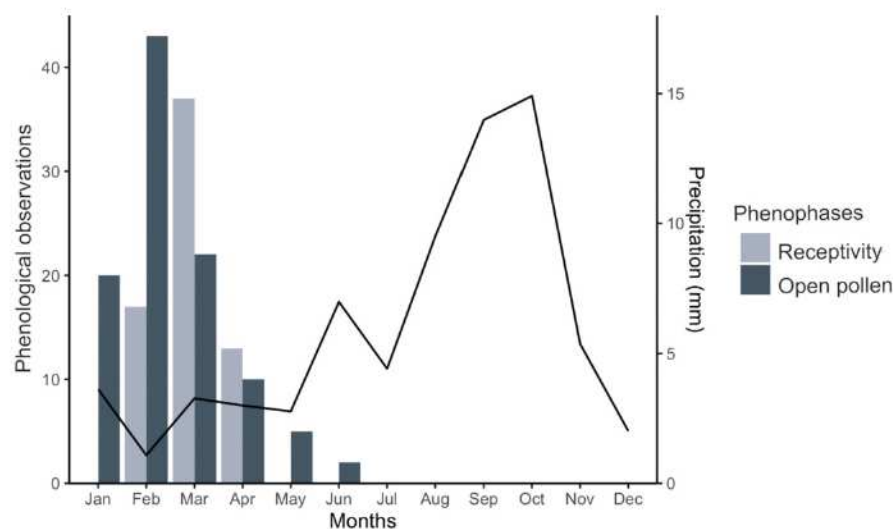


Table 2. Spearman correlation coefficients of phenophases with climatic variables (precipitation and temperature) for the three *Ceratozamia* species.

Phenophases	<i>C. brevifrons</i>		<i>C. delucana</i>		<i>C. moretii</i>	
	P	T	P	T	P	T
Receptivity	−0.3547	−0.3328	−0.5140	Null	−0.4497	−0.6504
Open pollen	−0.3197	−0.0779	−0.6121	Null	−0.7737	−0.6066

Note: P is average monthly precipitation and T is average monthly temperature.

ability of hybridization. More detailed studies on the pollinators of these species are required. Of particular interest is *C. tenuis*, another species that is found relatively near to our study area and in which more than one species of insect pollinator has been reported to be associated with its pollen strobili (Santiago-Jiménez et al. 2019). This suggests the possibility that pollinators may move pollen among species. Additionally, the same pollinator species has been found in up to four geographically close species of *Ceratozamia* — for example, the pollinators, *Ceratophila picipennis* (Tang, Skelley & Pérez-Farrera, 2018), and *Ceratophila chiapensis* (Tang, Skelley & Pérez-Farrera, 2018) are all found in strobili of *Ceratozamia vovidesii* Pérez-Farr. & Iglesias, *Ceratozamia alvarezii* Pérez-Farr., Vovides & Iglesias, *Ceratozamia mirandae* Vovides, Pérez-Farr. & Iglesias, and *Ceratozamia norstogii* D.W.Stev. (Tang et al. 2018). Thus, hybridization is a distinct possibility in these *Ceratozamia* species. However, because no hybrids have been reported in *Ceratozamia*, prezygotic or postzygotic or both, other isolation mechanisms could be involved.

Reproduction in *Zamia portoricensis* Urb. is influenced by the spatial distribution of the polliniferous and ovulate plants; however, reproductive success in this particular case is variable (Lazcano-Lara and Ackerman 2018). In addition, the close relationship between gymnosperm plants and their insect pollinators through mutualism, where both have dual benefits present challenges to our understanding of reproductive biology of cycads (Marler 2010; Salzman et al. 2021). Studies focusing on the nursery-deception pollination system of cycads and their phenological patterns provide a perspective into the evolution of this interaction, improving our understanding of long-term persistence of cycads. In turn, these patterns could enlighten how genetic exchange might occur over time under these conditions. We acknowledge the limitations of a five-year study window for investigating phenological patterns in cycads (Fig. 5); however, our results showed the relevance of inclusion of longer-term data for the study of reproductive dynamics in these gymnosperm taxa.

The influence of climatic variables in the phenological patterns observed in these *Ceratozamia* species should not be downplayed. The R and OP phases occurred during the dry season (Figs. 6–8). It is possible that heavy rains might inhibit pollination. The effect of rain on cross-pollination in several angiosperms varies and may be related to pollen resistance to water and (or) mechanical and energetic constraints of pollinators (Lawson and Rands 2019). In cycads, pollen grains are short lived (Chamberlain 1926), a condition which could hinder the arrival of viable pollen after storms. In addition, the DO phase occurred during the rainy season for the three species. Because seeds of *Ceratozamia* are suscep-

tible to desiccation, this pattern allows for the seeds to be moist for a longer period of time, thus preventing the embryo from desiccation. This is very necessary in *Ceratozamia* for the embryo to properly finish its development in approximately one year after the onset of the DO phase (Norstog and Nicholls 1997). This observed pattern in *Ceratozamia* differs from that recorded in species of other cycad genera — e.g., *Zamia boliviana* (Brongn.) A.DC., in which R and OP occur between August and November and maturation of seeds was recorded during the dry season (Segalla et al. 2021). Our results revealed that, in addition to precipitation, the temperature is also more constant during the months of the seed development in ovulate strobili. Even though these results were obtained from climate data from areas adjacent to the cycad populations, there is a different precipitation regime between their distribution ranges (Figs. 6 and 7). This approach indicates the need to directly collect climate variables at the sample sites. Overall, we suggest that more precision is required to evaluate the influence of climate in this phenological pattern, including other climatic variables (Renzi et al. 2019).

Considering the high extinction risk for cycad species, a better understanding of their phenological patterns would allow for a more refined evaluation of the anthropogenic activity-driven factors — such as habitat fragmentation and (or) size reduction of natural populations — that affect reproductive population dynamics (Morellato et al. 2016; Martínez-Domínguez et al. 2021). Ultimately, these data would be valuable to propose conservation strategies. It should also be noted that under adverse conditions such as fire, cycads could still generate viable strobili (Tang 1990). Ovulate plants do not produce strobili every year as a result of their longer lifespans, while polliniferous plants do so every year, or at least comparatively more often (Clark and Clark 1987; Pérez-Farrera and Vovides 2004; Calonje et al. 2011). These differences are related to the energy drain derived from producing many seeds, each with a well-developed megagametophyte, containing storage material for the germinated seed and seedling. It is common that some cycads do not even produce new flush of leaves in the particular season that they produce a strobilus (Stevenson 1981); thus, the energy drain on seed producing plants is considerably more than that for pollen-producing plants. That is pollen plants are transitory in reproduction; this is, in turn, related to differential resource investments in reproduction by pollen-producing plants (Calonje et al. 2011). Finally, the correlations in timing patterns between ovuliferous and polliniferous plants described in this study could serve as resources for future integrative biological studies in Neotropical cycads, particularly under climate

change scenarios, where the alterations in seasonal patterns could affect their population dynamics in relation to animal pollinators and, therefore, their mutual survival.

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Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Author contributions

L.M.D. collected data in the field, analyzed the data, and wrote the manuscript; F.N.M. collected data in the field and wrote the manuscript; F.V.S. and D.W.S. contributed to the revision and writing of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they do not have conflict of interest.

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