



## Research

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# Moonlight pollination in the gymnosperm *Ephedra* (Gnetales)

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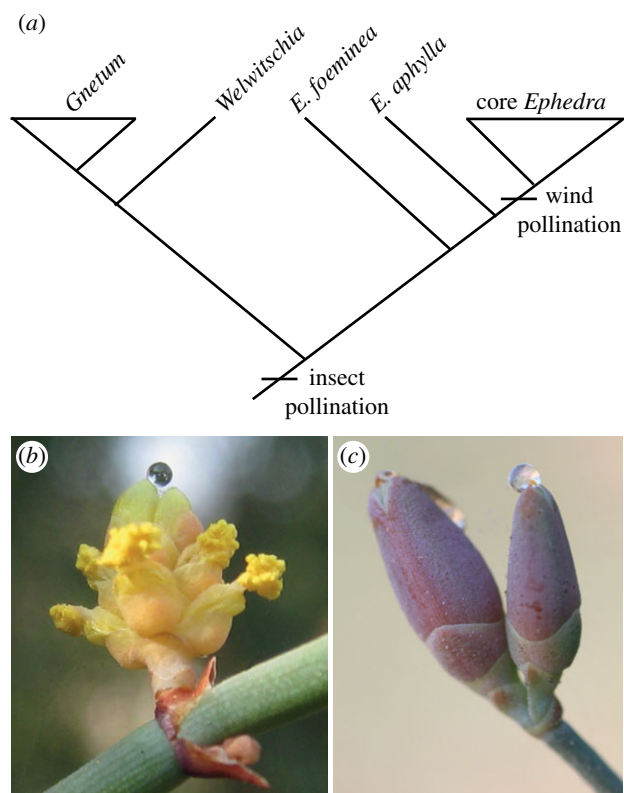
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Most gymnosperms are wind-pollinated, but some are insect-pollinated, and in *Ephedra* (Gnetales), both wind pollination and insect pollination occur. Little is, however, known about mechanisms and evolution of pollination syndromes in gymnosperms. Based on four seasons of field studies, we show an unexpected correlation between pollination and the phases of the moon in one of our studied species, *Ephedra foeminea*. It is pollinated by dipterans and lepidopterans, most of them nocturnal, and its pollination coincides with the full moon of July. This may be adaptive in two ways. Many nocturnal insects navigate using the moon. Further, the spectacular reflection of the full-moonlight in the pollination drops is the only apparent means of nocturnal attraction of insects in these plants. In the sympatric but wind-pollinated *Ephedra distachya*, pollination is not correlated to the full moon but occurs at approximately the same dates every year. The lunar correlation has probably been lost in most species of *Ephedra* subsequent an evolutionary shift to wind pollination in the clade. When the services of insects are no longer needed for successful pollination, the adaptive value of correlating pollination with the full moon is lost, and conceivably also the trait.

## 1. Background

The moon influences biological systems. Correlation with the lunar cycle has been described regarding activity, reproduction, communication and navigation for a number of vertebrates and invertebrates [1]. Even eyeless animals such as reef corals correlate their reproduction with the phases of the moon [2]. Therefore, it is not surprising that the moon can influence plants as well, e.g. as a consequence of plant–animal interactions. Pollination biology in *Ephedra* (Gnetales) has gained renewed interest since a recent study documents variation in pollination syndrome in this small gymnospermous relict [3], which can be traced back to the Early Cretaceous [4]. Insect pollination is conceivably the ancestral state in the Gnetales (figure 1*a*). The sister species of the remaining *Ephedra*, *Ephedra foeminea* [5], is insect-pollinated [3,8,9], as are *Welwitschia* [10] and *Gnetum* [11] (Gnetales), and probably also *Ephedra aphylla* [12]. By contrast, other species of *Ephedra* are considered wind-pollinated [13] although this has only been rigorously tested for *Ephedra distachya* [3,9]. As most gymnosperms, *Ephedra* produces liquid by secretion from the nucellus [6,14], and the liquid is exposed as a pollination drop at the micropylar opening (figure 1*b,c*). Its main function is to receive and transport pollen to the nucellus [14], but in the Gnetales, the pollination drops are high in sugar [15] and therefore attractive to insects.

The idea of a possible correlation between pollination and lunar phases in *E. foeminea* was founded owing to mistakes in our prediction of the time for pollination in this species. Considering the relatively stable Mediterranean climate, we had not anticipated substantial phenological variation, and we decided to go through all available data in order to find out why we repeatedly failed to predict pollination time in *E. foeminea*. The present study describes the results and conclusions.



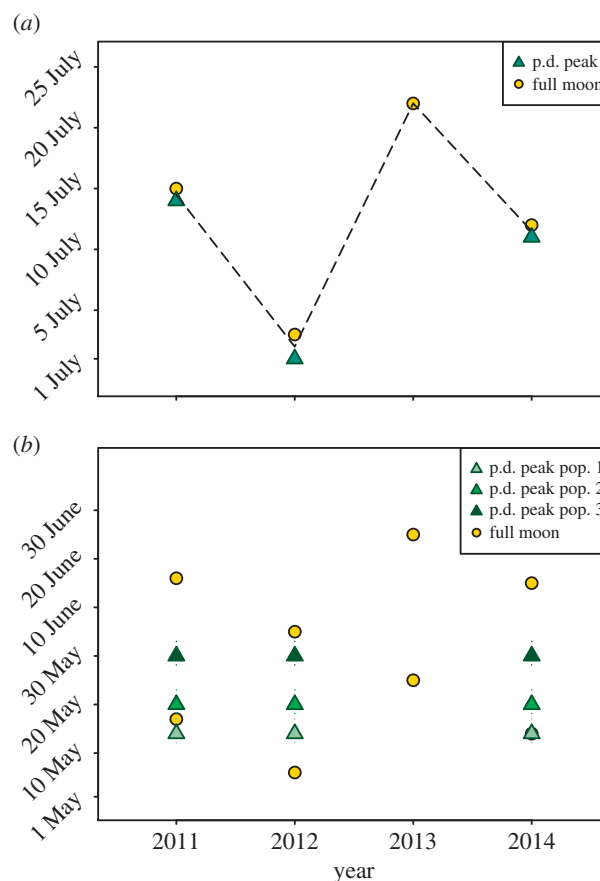
**Figure 1.** Pollination in the Gnetales. (a) Hypothesis on evolution of pollination syndromes in the Gnetales based on phylogenetic [5–7] and pollination [3,8–12] studies. (b) *Ephedra foeminea*. Male cone with pollination drops produced by sterile ovules at the distal centre of the cone. (c) *Ephedra foeminea*. Female cones with pollination drops exposed at the micropylar openings.

## 2. Material and methods

During 2011–2014, populations of *E. distachya* and *E. foeminea* were studied in Macedonia (Greece) and Dalmatia (Croatia). Pollination drop production and insect visitations were documented. Information on lunar phases was taken from [http://www.fullmoon.info/en/fullmoon-calendar\\_1900-2050.html](http://www.fullmoon.info/en/fullmoon-calendar_1900-2050.html). Correlation between the peak in the pollination period and the occurrence of full moon was assessed using regression analysis as implemented in R, v. 3.1.1 [16]. Temperature and precipitation for April–July 2011–2014 were extracted from <http://www.wunderground.com/history/>, compared using an ANOVA and assessed for correlation with pollination peaks as above. Additional details are available in the electronic supplementary material.

## 3. Results and discussion

The exact timing of pollination in *E. foeminea* varied considerably from year to year, but was correlated with the full moon of July (figure 2a) ( $r^2 = 0.999$ ,  $p = 0.013$ ). To our astonishment, even cones that appeared too young to be pollinated (small and ovules without developed micropylar tubes) secreted pollination drops from a pore-shaped micropylar opening during the peaking period at full moon. One to two weeks earlier, when the moon was new and in its first quarter, we observed the exact opposite; drop secretion was weak to non-existent and pollinators were absent. Not even cones of the appropriate developmental stage produced pollination drops. There are secondary peaks in pollination drop production in association with the full moon of August and September, but with few cones involved.



**Figure 2.** Correlation between peaks in pollination drop production and the full moon. (a) In *Ephedra foeminea*, the peak in pollination drop (p.d.) production occurs simultaneously in all study populations (green triangles) and is temporally correlated with the full moon of July (yellow circles). (b) In *Ephedra distachya*, the production peak occurs at different dates in different populations (triangles in different shades of green), and there is no correlation with the full moon (yellow circles).

Only a few studies in the literature provide the necessary details on dates and pollination drop production, but available information [8,12,17] supports our conclusions. Porsch [8] studied reproduction in *E. foeminea* in Dalmatia (Croatia) in late July to early August 1910. We have been puzzled by how late in the season he apparently found the plants at pollination stage of development, but in the light of the ‘lunar hypothesis’ presented here, Porsch’s [8] dates of observations make sense because in 1910, the full moon occurred on 22 July. The lunar hypothesis also explains why we failed to collect pollination drops during the first week of July in 2013 and 2014. The time should have been approximately right according to our observations from 2011 and 2012, and cones were indeed developed, but did not produce pollination drops. In 2014, drop production peaked simultaneously with the full moon of July (12th), as was also the case in 2011 (mid-July) and 2012 (first days of July). In 2013, our last observations from 10 July did not indicate a near start of the pollination period. Although impossible to know now, our guess is that the pollination drop production in 2013 peaked in association with the full moon on 22 July.

Our observations do not support a correlation between pollination and fluctuating weather conditions. The weather is stable in the Balkans; there are only rare exceptions to the otherwise warm and sunny summers with sparse precipitation

from May through to September (electronic supplementary material, figure S1). Furthermore, it is not the actual development of the cones that occur at different times or rates. Cones could be at pollination stage of development one–two weeks before the full moon, but did nevertheless not secrete pollination drops of sufficient amount to attract pollinators. Similarly, many cones were small and immature when the moon was full on 12 July 2014, and yet these cones produced pollination drops.

How then, could a correlation between pollination and the moon evolve? And how do the plants identify the lunar phases? Plants can detect moonlight [18], including its different intensities during the phases of the moon [19]. Plants can also detect gravity [20] and can conceivably use both to identify the lunar phases. Pollination in *E. foeminea* is a low-productive, generalist, entomophilous system [3]. We have identified pollinators of Diptera and Lepidoptera, several of them nocturnal. Nocturnal insects can fluctuate in abundance according to the phase of the moon [21–23], and some navigate using the angle (azimuth) to the moon [24,25]. Dung beetles use polarized moonlight to navigate in straight lines while moving dung [26]. At new moon or cloudy nights, they move around randomly [26]. Insect visitation of *E. foeminea* cones is not frequent [3] and pollinators caught have a limited number of pollen grains on their bodies. The consequence of poorly navigating pollinators may therefore be a costly deterioration of the pollination process. While some insects can navigate using land marks [27] or stars [25], navigation aided by moonlight is suggested to be widespread among nocturnal animals [21,26] and the system is probably particularly adaptive in open habitats [28]. This fits well with our field sites in the Balkans, where a cloudy sky is rare in the summer and no high trees shade the environment.

The moon may further be crucial for pollinator attraction. While investigated species of *Gnetum* emit easily detectable scent (C. Rydin 2014, personal observation and [11,29]), *Ephedra* does not. The colourful cones can attract diurnal insects but at night, field observations reveal only one possible means of attraction of pollinators: the many pollination

drops glitter like diamonds in the full-moonlight. A spectacular sight also for the human eye. The full moon is thus important for efficient pollination of *E. foeminea* both for pollinator navigation in the dark and for attraction to the cones. To experimentally remove the forces of celestial gravitation is difficult [30] but our hypothesis could be further tested by experiments on moonlight detection in plants and by continuing to make predictions followed by targeted observations [30].

By contrast, we find no correlation between the phases of the moon and secretion of pollination drops in the sympatric but wind-pollinated *E. distachya* (figure 2b) (May:  $r^2 = 0.34$ ,  $p = 0.39$ ; June:  $r^2 = 0.19$ ,  $p = 0.44$ ). Instead, its peak in pollination drop secretion occurs at approximately the same dates each year, on 20 to 30 May in Greece (somewhat later in France [17]). The time of the peak differs slightly between nearby localities, a difference that is constant between years and probably correlated with microclimate (see also [17]). Lunar-correlated pollination has probably been lost in *E. distachya* and other wind-pollinated species of *Ephedra*. Further studies of insect-pollinated taxa of the Gnetales (the tropical lianas of *Gnetum*, the Namib Desert endemic *Welwitschia* and the Mediterranean *E. aphylla* [10–12]) would, however, be highly interesting.

**Data accessibility.** The data reported in this paper are presented in the electronic supplementary material.

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**Author contributions.** C.R. conducted fieldwork, developed the hypothesis and wrote the manuscript with comments from K.B. K.B. conducted fieldwork, developed the hypothesis and performed statistical analyses with comments from C.R.

**Conflict of interests.** We have no competing interests.

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