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Research Article

Strobilus and seed production of *Dioon edule* (Zamiaceae) in a population with low seedling density in San Luis Potosí, Mexico

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Abstract. We describe strobilus and seed development in a *Dioon edule* (chamal, palma, dameu') population characterized by low seedling and high adult tree density, in order to improve conservation decisions for this endangered cycad species. Female strobili required 16-17 months and male 4-5 months to develop. During this period 80% female and 100% male strobili were not damaged by herbivores. The method of cone analysis used to evaluate seed production of pines was modified for *D. edule*, providing actual yield of individual strobili compared to potential seed yield, explaining productivity in terms of seed efficiency, and identifying and quantifying the types of seed failures. Larger strobili have higher seed potential calculated as the number of seeds potentially produced in the fertile megasporophylls of each strobilus, total seeds actually produced, and seeds with an embryo (filled). Calculated average seed potential per strobilus was of 230.4 seeds. Using the X-ray technique, average seed traits were 100.2 full, 1.8 insect damaged, and 0.6 malformed. Seed efficiency (number of filled seeds as a percentage of the seed potential of each strobilus) of 42.5% indicated that major seed loss was attributable to abortive ovules and seeds, possibly due to ineffective pollination. Seedling survival in greenhouse conditions was 100% after one year. Low seed production affects the population structure and hinders its conservation.

Keywords: Cycads, seed production, cone analysis, strobili, X-ray analysis.

Resumen. Se describe el desarrollo de estróbilos y semillas en una población de *Dioon edule* (chamal, palma, dameu') caracterizada por la densidad reducida de plántulas y elevada de plantas adultas, para tomar decisiones más acertadas sobre la conservación de esta especie amenazada. Los estróbilos femeninos requieren 16-17 meses y los masculinos 4-5 meses para desarrollarse. Durante este período, 80% de los estróbilos femeninos y 100% de los masculinos no fueron dañados por herbívoros. El método del análisis de conos usado para evaluar la producción de semillas de pino fue modificado para *D. edule*, informando de la producción real de semillas en cada estróbilo comparada con la producción potencial, explicando la productividad en términos de eficiencia de semilla e identificando y cuantificando los tipos de pérdidas. Los estróbilos más grandes tienen mayor potencial de semilla, calculado como el número de semillas producidas potencialmente en los megasporófilos fértiles de cada estróbilo, total de semillas efectivamente producidas y semillas con embrión (llenas). El promedio de semillas potenciales fue de 230.4 semillas. Utilizando la técnica de rayos X, el promedio de las variables de las semillas fueron 100.2 llenas, 1.8 dañadas por insectos y 0.6 malformadas. La eficiencia de semilla de 42.5% (proporción de semillas llenas en relación al potencial de semilla de cada estróbilo) indicó que la mayor pérdida de semilla es atribuible a los óvulos y semillas abortados, posiblemente debido a la inefectividad de la polinización. La sobrevivencia de las plántulas en invernadero fue de 100% después de un año. La baja producción de semilla afecta la estructura de la población y dificulta su conservación.

Palabras clave. Cícadas, producción de semillas, análisis de conos, estróbilos, análisis con rayos X.

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Introduction

The chamal or dameu' (*Dioon edule* Lindl., Zamiaceae) is a medium-sized cycad with an erect trunk and a rigid crown of long, light green leaves. It is endemic to Mexico, distributed along the Sierra Madre Oriental from southern Tamaulipas, through San Luis Potosí, Querétaro, Hidalgo and to central Veracruz [1]. Extensive collection of the plants and destruction of their habitat threaten their biological viability, and *Dioon edule* is therefore listed as an endangered species by the Mexican government [2] and as near threatened by IUCN [3].

These plants are important from an evolutionary standpoint because they belong to the group of gymnosperms [4], i.e., their reproductive structures are strobili or cones. They are strictly dioecious: when they mature, they produce male or female strobili. The basic structure is similar in both males and females, with a central axis and structures called sporophylls or scales [5]. Although female plants produce one strobilus each year, male plants can produce from one to four. The female strobili are larger than the males and bear the ovules that subsequently will be fertilized by pollen.

Studies conducted in populations of *Dioon edule* have shown that seed production is good, from 86 to 230 seeds per strobilus with75-90% germination [6, 7]. However, under natural conditions, the mortality at the seed and seedling stages is high (almost 90 %) [7] due to prolonged drought in the dry tropical forest habitat of *D. edule*, the recalcitrant nature of the seeds, predation by the field mouse *Peromyscus mexicanus*, and dehydration of seedlings during the first year [6-8].

Particularly in the region of the Sierra Madre Oriental in the state of San Luis Potosi, some populations of *D. edule* have densities of 775 to 3775 individuals ha^{-1} [9] and are currently threatened by prolonged drought, herbivory, fire, changes in land use, plunder of plants and seeds for illegal sale, and collection of the female strobili by some Xi'iuy indigenous communities who use the seeds in food preparation [10]. The differences in population density may be due to the number of germinated seeds and established seedlings in each population. A mortality rate of 86 to 98% was observed by counting the number of plants producing female strobili in each population and considering the average number of potential seeds produced in each strobilus [9]. Nevertheless, one particular population presented the lowest seedling density (112 ha^{-1}), 86% below the average (950 ha^{-1}), in contrast to the highest reproductive adult density (250 ha^{-1}), 66% above average [9]. We examined whether the low seedling density could be affected by low seed production and if so, what factors would be involved.

Various methods for estimating seed production have been developed, considering that annual seed production in plant species is variable and can be attributed to several factors. Particularly in conifers, cone or strobilus analysis developed by Bramlett et al. [11] is useful because it is one of the most efficient methods; it compares the actual seed production with the potential production of an individual strobilus, allowing productivity to be expressed in terms of seed efficiency of the strobili that reach maturity. This method has already been used to evaluate seed production and the magnitude of its loss in different coniferous species, and to identify the factors that affect their production [12].

A basic knowledge of strobilus and seed development and morphology is essential to understand this method of strobilus analysis [11]. There are several studies of cycads distributed in Mexico, describing strobilus and seed morphology [5, 7, 13], or including some aspects of strobilus phenology and seed production [6, 14] but few describing seed anatomy [15-17].

Assuming that *Dioon edule* seeds are produced in strobili through a process similar to that in conifers, we analyzed the seed production of one population with a high density of reproductive adults and low density of seedlings distributed in San Luis Potosi, by modifying the method of strobilus analysis. The objectives of this study were: a) to characterize the mature female strobili and seeds that *Dioon edule* produce, b) to quantify the seed loss and identify the responsible factors, and c) to estimate seed germination and seedling survival in greenhouse conditions.

Methods

Study area

The study area is located in a 1.35 ha ravine at 700 m a.s.l., with a semi-warm temperate climate, mean annual temperature of 23 °C, total annual precipitation of 1892 mm, calcareous and clayey soil, vegetation of submontane scrub and oak forest (*Quercus laeta* Liebm), surrounded by pasture lands. The population selected for the study has 250 reproductive adult plants ha^{-1}, with sex ratio of 1:2 male to female, and 112 seedlings ha^{-1}.

Strobili characterization

The observation of the visible development of 10 female and male strobili of *D. edule* in the study site began in July, 2007 and ended in November, 2008, when most seeds were dispersed. In August 2008, four male dehiscent strobili releasing pollen were collected and hermetically sealed in polyethylene bags to collect the insects that were between the microsporophylls at that moment, and the insects were subsequently identified. In May 2008, nine mature female strobili without apparent damage were selected, removed from the plant and transported to the laboratory (Mexican government authorization SEMARNAT SGPADGVS/00507/08). For each strobilus, the initial weight, length and apex, middle and base width were registered; they were subsequently stored at room temperature until the megasporophylls began to drop off at the end of 60 days, when the final weight of dehydrated strobili was recorded in order to measure the amount of water lost from harvest to dehiscence. The moisture content of the strobilus (%) was determined as ([final weight - initial weight] / final weight) x 100.

The megasporophylls were detached manually to identify their position along the strobilus and classify them according to Chamberlain [15] as follows: at the end of the strobilus base, there are one or two rows of yellowish or greenish megasporophylls that are glabrous and never bear seed primordia or ovules, followed by one or two rows of tomentose sporophylls that occasionally have ovules. At the apical end of the strobilus, the megasporophylls are densely tomentose, almost circular in their cross section, with two aborted ovules each, although those found in the lower rows bear ovules that sometimes mature into seeds. The sporophylls of the middle region are densely tomentose and contain two ovules that can develop into mature seeds if pollinated.

Ovules and seeds classification

The classification of ovules was performed using the characterization previously described by Chamberlain [15] as follows: the abortive ovules exist in each strobilus and fall into two classes: those that were not pollinated and those that were pollinated but did not develop due to a lack of space or poor nutrition. The ovules that have not been pollinated are ovoid, 8 mm or less in length and have a sclerotesta that is not rigid and a sarcotesta that is not smooth. In contrast, the pollinated ovules and abortive seeds are ovoid to flattened in varying degrees, are 15 mm or more in length, do not reach the normal size of mature seeds, and have a more rigid sclerotesta and a smooth sarcotesta.

The seeds of *D. edule* are generally described as ovoid to almost spherical in shape (3.0-3.5 cm length, 2.0-2.5 cm width) and sessile, although many seem stalked because they bend and emerge in a structure resembling a stalk at the sporophyll base during growth [15]. Three layers are distinctive in the integument: the fleshy outer layer called the sarcotesta, which is white or cream when unripe and yellow when ripe, the hard and stony middle layer called the sclerotesta, and the soft inner layer called the endotesta. The embryo is immersed in the female gametophyte and consists of two cotyledons, between which lie the plumule and hypocotyl, which extends up to the radicle, which is covered by the coleorhiza [17, 18].

A sample of ten seeds per strobilus, classified as filled seeds after X-ray analysis, was used to measure their length, width and weight.

X-ray seed analysis

The sarcotesta were detached from the developed seeds collected from each strobilus and were then fixed to an adherent, transparent and highly pressure-sensitive plastic sheet so that when tested with X-rays, the image would correspond to each seed. We used X-ray equipment Orthopantomograph OP 100 (Instrumentarium Imaging, Tuusula, Finland) at a power of 77 kV, an intensity of 12 mA, a focus-film distance of 120 cm and exposure time of 0.10 s without applying any contrast agent. The radiographic images of the seeds were analyzed with a lighted background and classified according to the appearance of the embryo and female gametophyte, with the radiographic pattern of seeds of *Ginkgo biloba* [19] as a reference as follows: a) full seeds, in which the embryo has completed development and is completely enveloped by the megagametophyte and is completely surrounded by the integuments, without any evidence of damage; b) empty seeds, which appear normal in external appearance but contain only a remnant of the embryo or megagametophyte; c) malformed seeds, which have an absent embryo or a deformed or incomplete gametophyte; d) seeds damaged by insects, in which the insect that feeds on the seed is present inside the seed or the damage they cause is detected and e) seeds damaged by fungus, recognized as dark or hazy areas in the megagametophyte [11].

Those seeds classified as damaged by insects were cut in half to inspect the internal damage, and further seeds with external damage similar to that observed for the insect collection were sectioned in the field.

Germination and viability test

The germination test was conducted on the 902 filled seeds, using a commercial germination mix (peat, perlite and limestone) in a greenhouse with an average annual temperature of 21 °C, a relative humidity of 73% and a maximum intensity of illumination at noon of 3799.78 lm m^{-2} during the winter and 9720.13 lm m^{-2} for the remainder of the year (daylight). Seedling survival was evaluated after 12 months [8]. The tetrazolium test was done in all ungerminated seeds left after the germination test to evaluate the embryo viability [20].

Strobilus analysis indicators

The key indicators for assessing the efficiency of seed production are [11]: seed potential or the potential of the strobilus to produce seed (number of fertile megasporophylls x two potential seeds); seed efficiency or the number of undamaged seeds with an embryo effectively produced compared to seed potential ([number of undamaged filled seeds / seed potential] x 100); extraction efficiency or the effectiveness of extraction procedure ([number of developed seeds extracted / total number of developed seeds produced by the strobilus] x 100); full seed germination (obtained from the germination test, tells how many of the developed seeds have the capacity to produce a seedling); and seedling efficiency or the proportion of the seed potential that is capable of producing seedlings (seed efficiency x extraction efficiency x germination percentage / 10000).

Statistical analysis

A correlation analysis using the Spearman coefficient test was performed to determine the relationship among female strobilus dimensions and magnitude and the characteristics of the abortive ovules and seeds. Basic statistics and correlation analysis were performed with MYSTAT12 for windows v. 12.02.00, by SYSTAT software, Inc.

Results

Female and male strobili

The male and female strobili of *D. edule* were observed from June–July, 2007 during the period when a small brown woolly dome (female strobilus) or a white pubescent dome (male strobilus) were formed at the center of the crown of leaves (Fig. 1a). In August–September, pollen was released from the male strobili with dehiscence of the microsporophylls, which starts at the base towards the apex, and the receptivity of the female strobili was demonstrated by infertile basal megasporophylls that were open (Fig. 1b). In both, the strobili were vertical, and insects were observed on the surfaces of the mega- and micro-sporophylls. In November-December, the male strobili had declined and were horizontal with dehydrated microsporophylls, and the female strobili were vertical and continued to increase in size and remain tomentose, with the basal megasporophylls already closed (Fig. 1c). In January-February, 2008, the male strobili were disintegrating but remained on the plant, and the female strobili were vertical, larger, and had closed megasporophylls (Fig. 1d).

In May-July, the basal or total microsporophylls were dehydrated in the male strobili in some plants, and the female strobili were vertical, with the megasporophylls separated mainly at the apex and seed development beginning to cause them to separate (Fig. 1e). In August-September, the female strobilus stalk elongated, making it lean, and the megasporophylls began to lose their woolly pubescence (Fig. 1f). In October-November, the female strobili were observed with megasporophylls that were detached from the axis, releasing the seeds to be scattered on the ground, although sometimes the lower third remained attached to the plant (Fig. 1g). On average, the time between the start of the formation of the female strobili until they are opened is between 16 and 17 months.

Each year different plants produce the strobili. In the months of June-July, plants initiate the female and male strobili, and at the same time there are plants with mature female strobili and the remains male strobili of last year. Similarly, in August-September, most plants are simultaneously present in states of male strobili pollination and female strobili receptivity and in a state of female strobili seed dispersion. At this time, female strobili in both states are damaged by herbivory. The caterpillars of the great cycadian butterfly *Eumaeus childrenae* G. Gray (Lepidoptera: Lycaenidae) occurred in large numbers on and between the basal megasporophylls, eating the tissue when the strobilus was receptive. An unidentified animal removed the megasporophylls of mature strobilus and carried off the seeds, but some of the seeds were left beside adult plants, showing that only the sarcotesta was consumed. At the end of the period, 80% of female and 100% of male strobili reached maturity.

The insects observed among the sporophylls of male strobili (n=4) at the time of pollen release (August–September) were the fire ants *Solenopsis* sp. (Hymenoptera: Formicidae), the weevil *Rhopalotria* sp. (Coleoptera: Belidae), the langurid beetle *Pharaxonotha* sp. (Coleoptera: Languriidae) and the orange-red beetle *Aulacoscelis vogti* Monrós (Coleoptera: Orsodacnidae). *Aulacoscelis* adult individuals were also observed on the megasporophylls of female strobili.

On mean ± S.E., female strobili (n = 9) had a length of 23.52 ± 0.73 cm and a diameter of 13.75 ± 0.42 cm at the base, 14.44 ± 1.33 cm in the middle and 9.82 ± 0.69 cm at the apex, and weighed 1.52 ± 0.19 kg at harvest. When the sporophylls began to detach from the axis, the strobili weighed 1.43 ± 0.18 kg, indicating that the moisture content loss was 6.15 ± 0.82% between when they were harvested and when the megasporophylls detached.

Fig. 1. The male and female strobili development of *Dioon edule.* a) Strobili initiation in June–July, b) pollen release and receptivity with insect visitation in August–September, c) microstrobilus declination and megastrobilus incrementing size in November–December, d) microstrobilus disintegrating and megastrobilus incrementing size in January–February, e) megastrobilus with separated sporophylls in May-July, f) megastrobilus lean and sporophylls losing pubescence in August–September, g) megastrobilus dehiscent releasing mature seeds in October–November next year.

The base of each megastrobilus (Fig. 2a) exhibited a mean of 8.4 ± 1.1 small, greenish, glabrous megasporophylls without ovules and 13.0 ± 1.2 tomentose megasporophylls (Fig. 2b) with some abortive ovules. There were 10.2 ± 1.0 tomentose sporophylls (Fig. 2c) with two abortive ovules in the apical region. The middle region showed an average of 115.2 ± 4.6 tomentose sporophylls (Fig. 2d) hosting two developed seeds, abortive seeds or abortive ovules.

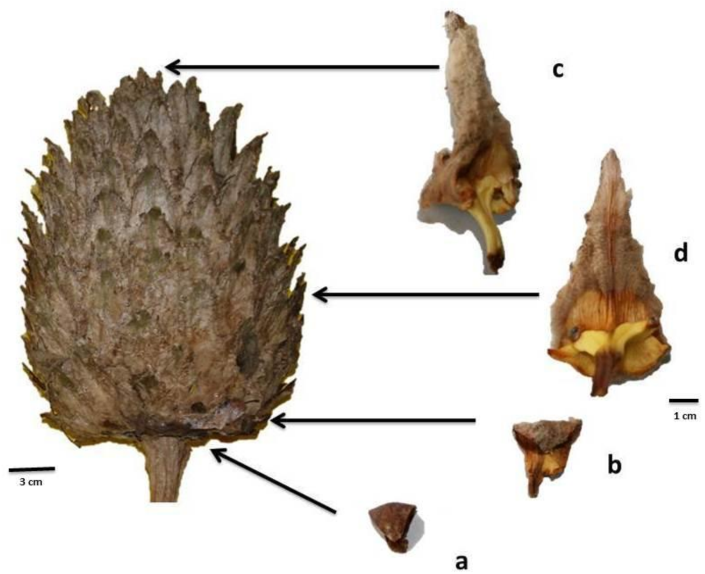

Fig. 2. Megastrobilus distribution of a) glabrous megasporophylls in the basal portion, b) tomentose megasporophylls in the base, c). tomentose megasporophylls in the apical portion, and d) tomentose megasporophylls in the middle portion, bearing seeds or ovules.

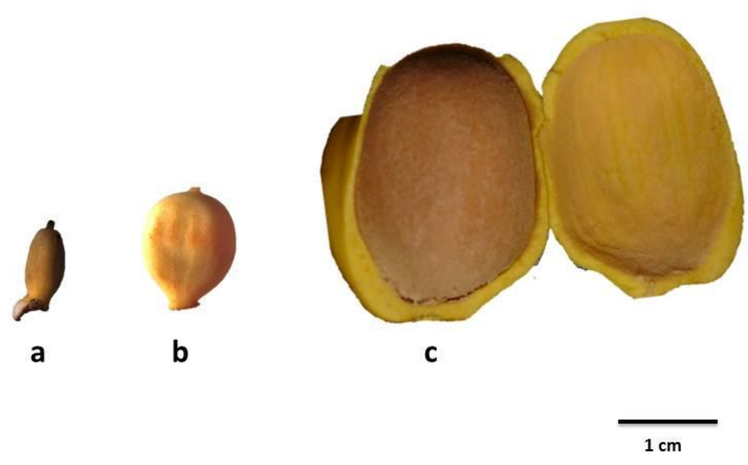

Fig. 3. Classification of *Dioon edule* Lindl. a) abortive ovules, b) abortive seeds, and c) developed seeds.

Ovules and seeds

After removing the megasporophylls, we detected a mean of 93.8 ± 25.3 abortive ovules (Fig. 3a), 34.1 ± 25.3 abortive seeds (Fig. 3b), and 102.6 ± 25.8 developed seeds (Fig. 3c) per strobilus. Radiographic imaging allowed us to classify and count the developed seeds as: full (Fig. 4a), malformed (Fig. 4b), and damaged by insects (Fig. 4c). The distribution of these seed types is given in Table 1, and there were no empty or fungal-damaged seeds. Insect damage in the seeds corresponded to the *Pharaxonotha* sp. beetle, which consumes the embryo tissue and completes its development inside the seed (Fig. 4d), building a gall and leaving behind outlets (Fig. 4c).

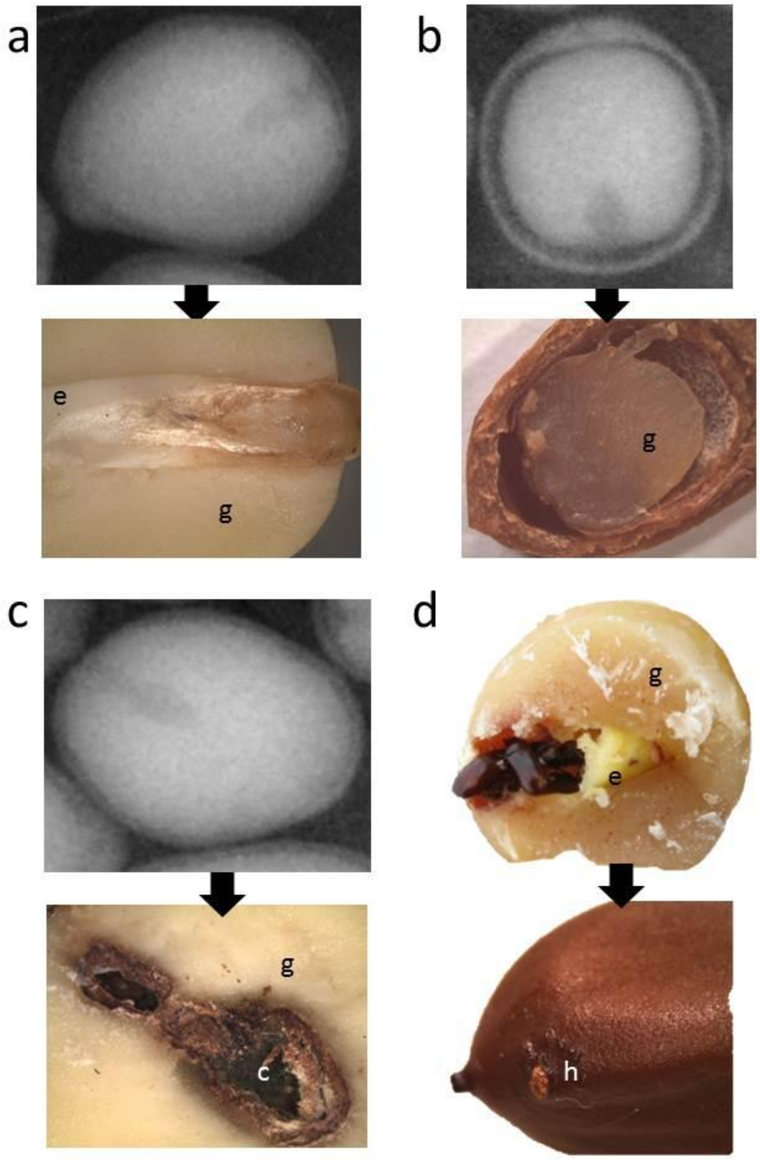

Fig. 4. Radiographic imaging of seeds classified as a) full; b) malformed; c) damaged by insects; d) *Pharaxonotha* beetle feeding on embryo tissue (photo by Alberto Prado) and insect exit hole. e = embryo; g = gametophyte; c = embryo cavity; h = exit hole.

Mean full seed length was 2.32 ± 0.37 cm, diameter was 1.89 ± 0.34 cm and weight was 4.14 ± 0.38 g. All the full seeds reached a germination rate of 23.84% after eight months. None of the seeds that did not germinate were viable after tetrazolium testing. A year after germination 100% of the seedlings survived in good condition.

Table 1. Classification of developed seeds per strobilus in *Dioon edule* (average ± S.E.).

Seeds	**Number**	**(%)**
Full	100.2 (± 30.4)	97.72
Insect damaged	1.8 (± 1.3)	1.73
Malformed	0.6 (± 0.6)	0.54

Strobilus analysis

The mean seed potential per strobilus was 230.4 ± 9.1 seeds, seed efficiency was 42.5 ± 12.4%, extraction efficiency was 100 ± 0.0%, seedling efficiency was 10.0 ± 4.7%, and seedling survival after 12 months was 100 ± 0.0%.

Female strobilus-seeds relationship

The largest negative association was found between the number of abortive ovules and the diameter of the base, middle and apex of the strobilus. Furthermore, there is a positive association between total seeds and number of full seeds, and the base and middle diameter of the strobilus, as well as between the seed potential and weight (Table 2).

Table 2. Spearman correlation analysis between strobili and seed traits.

	Megastrobilus				
	Weight (kg)	Length (cm)	Base width (cm)	Middle width (cm)	Apical width (cm)
Seed potential	0.693*	0.546	0.125	0.235	0.042
Total seeds	0.627	0.653	0.783*	0.828*	0.647
Full seeds	0.628	0.653	0.783*	0.828*	0.647
Abortive ovules	-0.281	-0.525	-0.946**	-0.908*	-0.810*

* < 0.05 **< 0.0001

Discussion

The production cycle of female strobili in the population of *D. edule* in San Luis Potosi is similar to that found for the species in Veracruz [6], *D. caputoi* in Puebla [13], and *D. merolae* in Chiapas [21,22]. In the population analyzed in this study, 16 to 17 months elapsed from strobilus emergence to dehiscence, with initiation occurring some months before June–July. The period of female strobili receptivity was August-September, and as reported in other *Dioon* species would last 10 to 21 days [4].

The *D. edule* male strobili take three to four months from emergence to pollination. However, Lázaro-Zermeño et al. [22] mention that *D. merolae* male strobili take seven months from emergence to reach full development. When mature, the strobilus elongates very fast, separating the sporophylls and releasing the pollen from the microsporangia [23]. Pollen release from male strobili occurs in August–September, with a one-month difference compared with the same species in Veracruz, which occurs in September–October [16] and in March–April for *D. merolae* [4]. This suggests that pollination could be related to environmental causes such as temperature and precipitation.

These results are consistent with those of a detailed study of models describing *Cycas micronesica* strobili development showing how the shape and timing of strobili expansion are relatively independent of location and season [24], and suggest that probably this is happening in some *Dioon* species.

Cycads maintain parasitism (herbivory) and mutualism (pollination) with specific herbivores that uptake and sequester the plant's toxic azoglucosides and neurotoxic nonprotein amino acids [25]. The *Eumaeus* larva feeds exclusively on cycads, and larva and older caterpillars of *E. childrenae* have been observed feeding on leaves and strobili of *Dioon edule, D. merolae*, *Ceratozamia matudai*, *C. mexicana*, *C. norstogii*, *C. robusta*, *Zamia fischeri* and *Z. soconuscencis* [26, 27]. In the study site, *E. childrenae* affected 10% of megastrobili in the initial stage of development, although this is not a common event [22].

Some birds and mammals feed on cycad strobili and later disperse the seeds [23]. In the study site some strobili were damaged by unidentified animals that feed on mature seeds. Previous studies reported that *Dioon edule* seed predation by the rodent *Peromyscus mexicanus* is up to 40% after dispersal, despite the seeds' toxicity, contributing to germination when the seeds are left in rodent dens with favorable environmental moisture and soil fertility conditions [6, 26, 28]. However, more studies quantifying strobili specific seed predation at the study site are needed.

Cycad pollination may occur by a combination of wind and insects [22, 29], but the insects observed in the male strobili are cycad specific pollinators and obligate cycad feeders [25]. When the strobilus shades pollen at maturity stage, thermogenesis occurs and heat production generally volatilizes odors attractive to insect pollinators [23]. Pollination by beetles is well documented in cycad species [30-33] where *Pharaxonotha* is the most important. It has been observed feeding on cycad pollen, in the sporophylls, axis and stem of the male strobilus, as well as on the megasporophylls when the female strobilus is receptive [8]. *Rhopalotria* are specifically associated with *Dioon* and are required for seed production [30]. Small numbers of *Aulacoscelis* were observed on the leaves and on the male and female strobili of *D*. *edule* in San Luis Potosi in August–September during the rainy season [32], similar to *D. merolae* seeds predated by *Aulacoscelis melanocereus* [21] and other species [33]. The presence of large numbers of ants (*Solenopsis* sp.) in the male strobili may be related to presence of mucilage [22], pollen, or insect predation because this ant is an opportunistic omnivore, but pollination by ants is rare [34].

The dimensions of the mature female strobili are in the lower limits of the range defined for the species of 20-35 cm length and 12-24 cm diameter [13, 27], although their populations are known to be highly variable and the forms found in the state of San Luis have female strobili that are 14 cm in length. Their average weight was below that reported for *D. edule* in Veracruz, which ranged from 2.5 to 5.0 kg [7] and *D. merolae* in Chiapas with an average of 3.6 kg [22]. The average number of potential seeds per strobilus was similar to the number of 230 seeds observed in Veracruz [7], suggesting that mature megastrobili of *D. edule* in this population of San Luis Potosi are similar to those populations in southern latitude.

Studies in conifers confirm that there are more seeds when the strobili are large and heavy [35], consistent with the correlation analysis of *D. edule* in the study area, because larger megastrobili have more seed potential, total seeds and filled seeds. These observations suggest that when there are more fertile sporophylls with the potential to produce more seeds, the weight of the strobilus increases. Additionally, when there are fewer abortive ovules and more developed seeds, the strobili are wider in the middle portion.

The loss of moisture in the strobilus was very small compared to conifers with woody strobili such as *Pseudotsuga menziesii*, which lose up to 80-85% before releasing the seed [36]. *Zamia,* and probably also *Dioon,* require many months of warm stratification before germination because they have non-deep simple morphophysiological dormancy [37].

With an average seed potential per strobilus of 230.4, 21.5% of megasporophylls were infertile and 78.5% were potentially fertile, implying a good level of seed production. However, the seed efficiency was 42.5%, which is very small compared with *D*. *califanoi* in Oaxaca, which has a seed efficiency of greater than 70% [38]. The average production of developed seeds by the strobilus is less than the 228 seeds per strobilus of *D. edule* in Veracruz [18], and is similar to the 111 seeds per strobilus of *D*. *caputoi* in Puebla [14], although the presence of abortive ovules or seeds is not mentioned for the other species.
Considering that fewer than 2% of the seeds were damaged by insects, low seed efficiency value may be the result of the elevated number of ovules that were not fertilized. The presence of this type of ovule had been mentioned for *Cycas circinalis*, including ovules that shrink and change to appear black in color [39]. It has been suggested that the high proportion of unfertilized ovules (50-100%) may be indirect evidence of insufficient pollen production to fertilize the ovules; of lower probability that female strobilus matures simultaneously with available males; of longer distance between female and male strobili; and/or of the low efficiency or absence of the pollinators activity [4,15, 21]. These conditions could be occurring in the study area because the population is surrounded by pastures with considerably disturbed vegetation [9], and a sex ratio of 1:2 male to female. The sex ratio is 3:1 for *Dioon* species to ensure abundant pollen during female strobili receptivity [4].

The study site has been severely affected by the replacement of forests with pastures, and livestock owners generally try to remove the plants of *D. edule* so their cattle do not eat the leaves in the dry season, when they generally are poisoned and die, causing significant economic losses. It is unclear why the sex ratio is different from other populations, because the removal of plants is apparently not selective but is systematically related to the places where livestock graze.

Abortive seeds were not a common event, and the lack of development even when they were fertilized may be due to a less developed vascular system, although the causes are unknown [15]. The proportion of full seeds was higher than the proportions of seeds damaged by insects and malformed, which were very small. *Pharaxonotha* sp. is a beetle known to feed on cycad pollen, seeds and stored flour [30, 40, 41], and therefore, it is not rare that they feed on the embryo.

The seed extraction efficiency was very high because no seeds were fused. In the field, however, the lower base of the female strobilus often does not disintegrate, the megasporophylls remaining attached to the axis without dispersing the seeds.

The efficiency of seedlings was very low, as it is usually considered to be 50% when the objective is the production of seeds [11]. Nevertheless similar values have been observed in other endangered endemic species in Mexico with small populations, such as *Pinus ayacahuite* (15.77%) [42] and *Abies religiosa* (37.82%) [35]. Low germination caused by unidentified factors would explain this result, since low availability of viable seeds is present also in *D. merolae* [21], but reduced viability of seeds suggests dehydration of the gametophyte as one factor. In the natural habitat, 36% *D. edule* seedling mortality after one year has been observed [6], but in the greenhouse 100% of seedlings survived with constant irrigation and shadow.

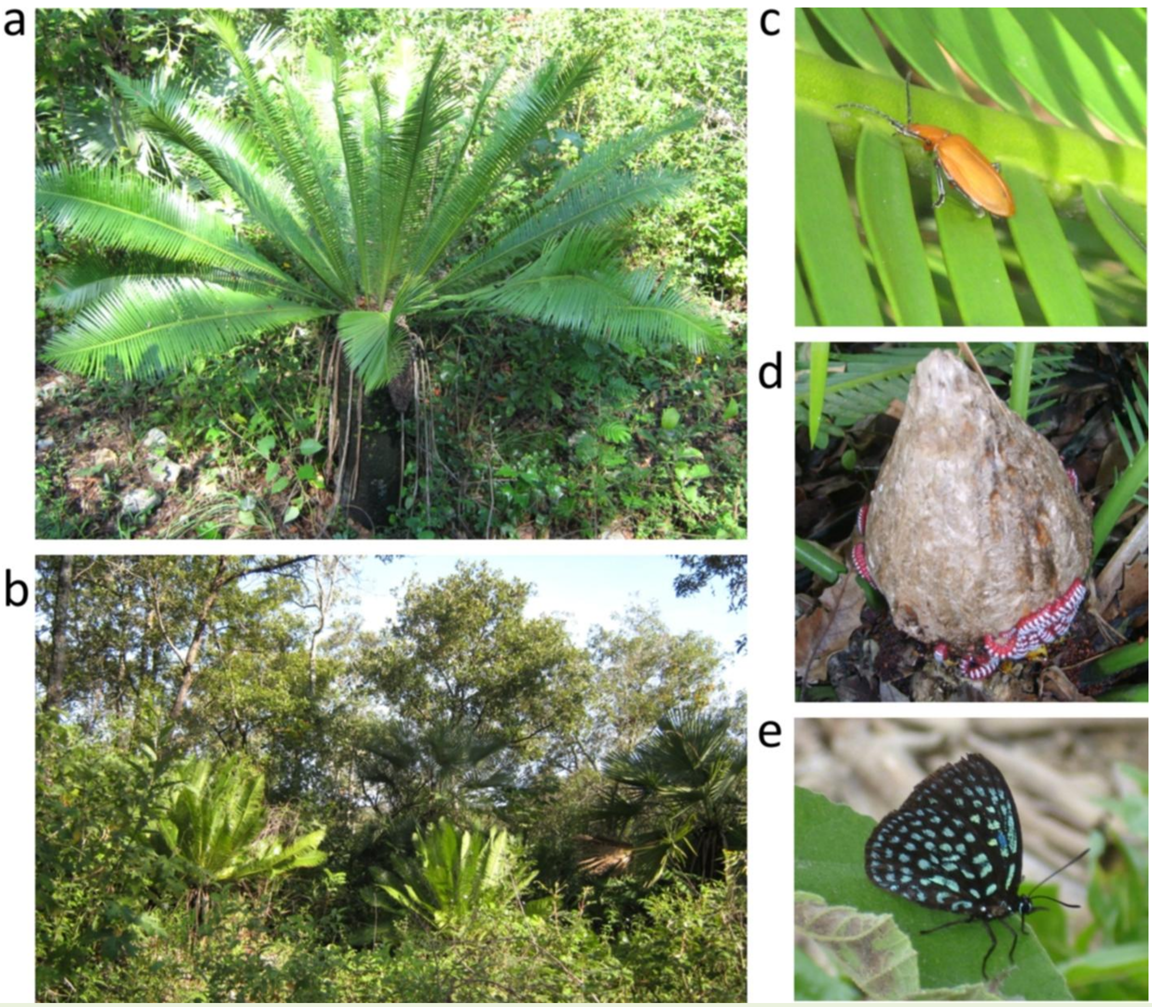

Fig. 5. Traits of chamal or dameu' (*Dioon edule* Lindl.) a) adult plant with an erect trunk (1.2 m) and a crown of long leaves; b) habitat in the submontane scrub and oak forest; c) the orange-red beetle *Aulacoscelis vogti* Monrós; d) caterpillars of the great cycadian butterfly *Eumaeus childrenae* G. Gray; e) adult of the great cycadian butterfly.

Implications for conservation

This population of chamal (*Dioon edule*) in San Luis Potosí (Fig. 5a,b) showed that male and female strobili development and seed potential are similar to populations of *Dioon* species in other locations. We recommend using the approach of Marler and Dogol [24] to carefully analyze the strobili expansion dynamics. Major seed loss was attributable to abortive ovules and seeds, possibly due to unsuccessful pollinators, lack of synchronicity in the pollination-receptive process of strobili, low sex ratio male:female (< 3:1), or excessive distance between male and female strobili. Plant-insect mutualism must be examined in the study area, because is relevant to long-term viability of the population, and strobili should be a protected habitat for insects to feed and reproduce (Fig. 5c-e). High density of reproductive adults does not guarantee natural recovery of the population because of the reduced density of female plants. All these factors must be considered when fecundity of *D. edule* is calculated for more accurate population ecology studies. X-ray image analysis has potential to be used as a rapid and nondestructive test to predict seed viability in the endangered *D. edule*.

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