Morphological Seed Traits and Germination of Six Species of Pachycereeae (Cactaceae)♦

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ABSTRACT

Morphological seed traits and germination responses of freshly matured seeds of six species of Pachycereeae: *Escontria chiotilla*, *Myrtillocactus geometrizans*, *Neobuxbaumia mezcalaensis*, *N. multiareolata*, *Pachycereus grandis*, and *Stenocereus queretaroensis* were studied at room temperature in laboratory conditions with the hypothesis that seed size affects germination. Seeds are different in shape and testa structure. Both, number of seeds per fruit and seed traits (length, width, width/length ratio, and mass) showed statistically significant differences. The highest correlation coefficients corresponded to seed length and width, length and mass, and width and mass. Seed germination started 3-6 days after sowing in those species with larger size and mass, whereas the smaller and lighter seeds of *E. chiotilla* and *M. geometrizans* required 15 days suggesting a delay in germination, thus seeds were nondormant. Seed size and germination response differences agree with hypothesis of appertaining to different phylogenetic clades.

Keywords: *Escontria*, *Myrtillocactus*, *Neobuxbaumia*, *Pachycereus*, *Stenocereus,* seed dormancy, seed mass, seed size.

INTRODUCTION

The family Cactaceae is distributed mainly in the arid and semiarid zones of North and South America. The Pachycereeae, one of the nine tribes of the subfamily Cactoideae, includes the columnar species that are mainly distributed in North America and the most species are located in Mexico (Dávila-Aranda *et al*., 2002). Cacti have been exploited since pre-Colombian times (Luna and Aguirre, 2001); for example, the fruits of columnar cacti, mostly of wild plants are traditionally used by local people from Mexico, Central America and northern South America. Some fruits are consumed for their delicious taste and because they contain secondary metabolites (flavonoids, terpenes, betalaines), many of them cause benefic effects to health, especially antioxidant action or by nutraceutical properties (Nerd *et al*., 2002; Esquivel, 2004; Emaldi *et al*., 2006). Among the columnar cacti, *Stenocereus queretaroensis* is the most cultivated and

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the commercially most important species of the genus, although the growth area is restricted to central Mexico (Nerd *et al*., 2002). *Escontria chiotilla*, as well, is a resource native to south Mexico mainly in the semiarid Mixteca region in Oaxaca, Mexico (Arellano and Casas, 2003). Medium to large edible fruits are produced by columnar species included in this study.

Seeds in the Cactaceae family show considerable variation in shape, size, and testa structure and colour (Barthlott and Hunt, 2000). Few studies have documented seed number per fruit in Cactaceae, which varies among species (Rojas-Aréchiga and Vázquez-Yanes, 2000; Godínez-Álvarez *et al*., 2003) and within individuals of the same species (León de la Luz and Domínguez-Cadena, 1991; Ayala-Cordero *et al*., 2004; Sánchez-Salas *et al*., 2006).

Moreover, studies on seed longevity, viability, and germination in Cactaceae have been published for few species (Godínez-Álvarez and Valiente-Banuet, 1998; Bowers, 2000; Rojas-Aréchiga and Vázquez-Yanes, 2000; Flores and Briones, 2001; Rojas-Aréchiga *et al*., 2001; De la Barrera and Nobel, 2003; Ayala-Cordero *et al*., 2004). The first reports concerning seed germination in this family came from the studies of Alcorn and Kurtz (1959) and McDonough (1964) who demonstrated that light has a stimulating effect on seed germination. Other studies have focused on temperature requirements (Rojas-Aréchiga *et al*., 1998) or a combination of light and temperature fluctuations to evaluate how they restrict seed germination in several Mexican species (Martínez-Holguín, 1983; Romero-Schmidt *et al*., 1992; Vega-Villasante *et al*., 1996; Rojas-Aréchiga *et al*., 1997; Ruedas *et al*., 2000; Flores and Briones, 2001; Ramirez-Padilla and Valverde, 2005). More recently in addition to light and temperature, the effect of water potential at different times after seed ripening has been tested (De la Barrera and Nobel, 2003; Ramírez-Padilla and Valverde, 2005). Knowledge on morphological traits of seed and their seed germination requirements is important to understand the possible role of different environmental factors on this critical phase of plants life-history. Cacti establishment from seed seems to be infrequent for some species, taking place mainly during favourable years (Rojas-Aréchiga *et al.,* 1997).

The purpose of this study was to determine whether freshly matured seeds have different morphological traits (shape, number of seeds per fruit, mass, and size) in six species of columnar cacti and whether their size has an effect on the germination response under uncontrolled laboratory conditions.

MATERIALS AND METHODS

Seeds were obtained from four ripe fruits collected from three individuals per species from their native populations (Table 1). The studied species *Escontria chiotilla* (F.A.C.Weber) Rose, *Myrtillocactus geometrizans* (Mart.) Console, *Neobuxbaumia mezcalaensis* (Bravo) Backeb., *N. multiareolata* (Dawson) Bravo, *Pachycereus grandis* Rose, and *Stenocereus queretaroensis* (Weber) Buxb. represented four monophyletic clades within the Pachycereeae tribe (Terrazas and Loza-Cornejo, 2002; Arias *et al*., 2003). Fruits were dissected in the laboratory to obtain their seeds, which were washed in tap water eliminating pulp remains and mucilage. Seeds were immediately placed in absorbent paper until they dried. The total number of seeds per fruit for each species was counted. To evaluate seed mass 25 seeds of each fruit were weighed individually (n = four fruits per individual) using an analytical scale (Scientech SA120). The same weighed seeds were used to measure their length and width, and from these measurements we calculated the roundness index (width/length ratio). Other features, such as seed shape and testa colour and structure, were described using a dissecting microscope adapted to an image analyser (Media Cybernetics, 1997) following the terminology of Barthlott and Hunt (2000). For scanning electron microscopy (SEM), 3 or 4 seeds per species were washed using ultrasound and 95% ethanol. Dry seeds were fixed to aluminium specimen holders with double-slide adhesive tape and coated with gold in a JEOL-JFC-1100 sputter coater. Morphological observations and micrographs were carried out in a JEOL-JSM-5310LV field-emission SEM.

For seed germination three replicates of 50 seeds each, collected 5-10 days before starting germination, were used and placed under laboratory conditions. Seeds were disinfected by immersion in a solution of 10% commercial Clorox for 5 min (Vega-Villasante *et al*., 1996) and placed in distilled water for 24 h to remove the mucilage that remained strongly attached to the seed testa. These seeds were immediately sown on saturated filter paper with 10 ml of distilled water in 9 cm diameter sterile plastic Petri dishes. Petri dishes subsequently were wrapped with plastic film to reduce water loss. Germination was recorded at 3-day intervals for 30 days, when germination ceased. A seed was considered to be germinated when the radicle protruded. The accumulated germination percentage for each species was calculated by adding the observed percentage every other third day. Mean temperature in the laboratory during the day was 25°C, which has been reported to be optimal for seed germination of cacti (Nobel, 1988; Rojas-Aréchiga and Vázquez-Yanes, 2000), and it was 12°C during the night. Photosynthetic photonic flux density, as measured with a LI-COR photometer (LI 185A), was 50 µmoles $m^{-2}s^{-1}$, with a period of 12 h light.

Interspecific differences in seed size and mass were evaluated through variance analyses followed by a Tukey's pair-wise means comparison analysis ($P < 0.05$), whereas differences in number of seeds per fruit were evaluated through covariance analysis. The association among characters was detected through Pearson correlation analysis. Differences among cumulative percentages of seed germination curves among the six species were analysed using the LIFETEST procedure followed by paired comparisons between species germination curves using the same procedure where the six species were the strata (Fox, 1993; Allison, 1995). In both analyses the default Kaplan-Meyer estimator method for cumulative seed germination curves was used. The null hypotheses of equality of seed cumulative germination among and between species were tested by the log-rank or Wilcoxon tests (Fox, 1993; Allison, 1995). These analyses compare the observed cumulative number of germinated seeds occurring at each particular time with the number to be expected if the germination function of the species is the same. The log-rank test was used in cases where germination function of two species do not cross and Wilcoxon test where curves do cross (Geoff and Everitt, 2002). All analyses were performed with SAS software (SAS Institute, 1989).

RESULTS

Morphological Seed Traits

Seed shape and testa colour and structure were different among the studied species (Fig. 1). The almost round, small, black, dull, and rough seeds of *Escontria chiotilla* and *Myrtillocactus geometrizans* differed from the reniform, extremely large, black, glossy, and smooth seeds of *Pachycereus grandis* or the reniform, large, brown-black, glossy, and smooth seed of *Neobuxbaumia mezcalaensis* and *N. multiareolata*.

Neobuxbaumia mezcalaensis and *Stenocereus queretaroensis* had the highest number of seeds per fruit, whereas the lowest difference was for *M. geometrizans* fruits (Table 2). MANOVA detected significant differences for number of seeds per fruit among all species studied ($F = 891.4$, d.f. = 5, $P < 0.0001$, Table 2).

Seed mass also varied among the six species (Table 2). *Escontria chiotilla* and *M. geometrizans* had the lightest seeds and *P. grandis* the heaviest. Variance analysis revealed significant differences for seed mass among species ($F = 410.29$, d.f. = 5, $P < 0.0001$), except between *E. chiotilla* and *M. geometrizans* and *N. mezcalaensis* and *N. multiareolata* (Table 2).

Myrtillocactus geometrizans had the smallest seeds and they tended to be almost round as indicated by their roundness index (Table 2). *Pachycereus grandis* had the largest seeds and *S. queretaroensis* had seeds with the smallest roundness index. Variance analysis showed significant differences among the species for seed length (F = 353.97, d.f. = 5, $P < 0.0001$), width (F = 281.96, d.f. = 5, $P < 0.0001$), and roundness index (F = 31.69, d.f. = 5, $P < 0.0001$). Significant differences were consistently detected among species for both seed length and width (Table 2). However, differences in roundness index were only detected for *M. geometrizans*, *P. grandi*s and *S. queretaroensis* (Table 2).

Seed mass and seed size association among the six species showed significant correlations only between three pair-wise associations. The highest correlation coefficients were for length vs. width (r = 0.97, $P < 0.0001$, n = 1800), length vs. mass (r = 0.90, $P < 0.0001$, n = 1800), and width vs. mass (r = 0.87, $P < 0.0001$, n = 1800). At the intraspecific level, there were significant correlations between length and width for the six species studied (Table 3); however there were no significant correlations between length vs. mass, width vs. mass, or mass vs. number of seeds per fruit for all species (Table 3). *Pachycereus grandis* had the highest correlation coefficient between seed length and mass and between width and mass, whereas *S. queretaroensis* had the highest correlation coefficient for length vs. width (Table 3).

Seed Germination

Seed germination in most species was a rapid process and started 3-6 days after sowing (Fig. 2). In both species of *Neobuxbaumia* and *S. queretaroensis*, maximum germination percentage occurred 6 days after sowing, in *E. chiotilla* 15 days, and in *M. geometrizans* 18 days after sowing (Fig. 2). *Pachycereus grandis* had the higher percentage of germination at 14 days, although germination started 6 days after sowing (Fig. 2). Figure 3 shows the different cumulative germination functions for the studied species. The statistical analyses showed that cumulative germination functions were significantly different among the six species (Wilcoxon test: $\chi^2 = 566.29$ d.f. = 5, $P < 0.0001$). Moreover, with the exception of *P. grandis* and *S. queretaroensis*, paired comparisons of germination curves showed significant heterogeneity between corresponding paired cumulative germination curves (Table 4).

DISCUSSION

Morphological Seed Traits

Cactaceae seeds show considerable variations in their shape, size, and testa structure and colour. The species studied here were examples of this variation in seed shape and testa colour. The differences found in shape and testa traits supporting previous reports for species of these genera (Barthlott and Hunt, 2000; Arias and Terrazas, 2004; Arroyo-Cosultchi *et al*., 2006). According to Rojas-Aréchiga and Vázquez-Yanes (2000), the number of seeds per fruit in Cactaceae is highly variable. Some species may have more than 1000 seeds per fruit, whereas no more than five are present in others. In the studied species, *M. geometrizans* possessed 93-184 seeds per fruit and *S. queretaroensis* 435-1115 seeds per fruit; whereas, other species like *N. mezcalaensis* possessed an intermediate seed set per fruit. These differences may represent different strategies of the reproductive efforts of these species as mentioned by Harper *et al*. (1970). Moreover, studies on the interaction among size, shape, and number of seeds per fruit have shown that, undoubtedly, ovary size and, consequently, fruit size may sometimes restrict the space in which the seeds are developed (Harper *et al*., 1970). This could be the case in the studied species, since interspecific differences in their fruit size were evident (Bravo-Hollis, 1978; Arias *et al*., 1997; Pimienta-Barrios, 1999). Number of seeds per fruit may also depend on plant age and size as well as on number of flowers produced per plant as has been shown for *S. gummosus* and *S. thurberi* (Parker, 1987; León de la Luz and Domínguez-Cadena, 1991).

Other contrasting differences among the studied species corresponded to seed size and mass. Arias *et al*. (1997) mention an average seed length of 1-2 mm for *M. geometrizans* and *E. chiotilla* growing in the

Tehuacan Valley, and in our study, seeds showed similar values even when they were collected in different areas of Mexico, indicating their constancy. For the largest seeds of *P. grandis,* lengths of 5-6 mm have been reported (Arias and Terrazas, 2004) and a similar size was observed in this study. This was not the case for *N. mezcalaensis* with a seed length (4 ±0.29 mm) larger than that reported by Arias *et al*. (1997) for the population of Tehuacan Valley, suggesting the need to evaluate seed size in others. At intraspecific level, seed roundness index is a constant trait in *E. chiotilla*, *M. geometrizans* and *S. queretaroensis* given that in these species significant differences were not observed among individuals. The roundness index allowed us to express seed shape (Harper *et al*., 1970; Barthlott and Hunt, 2000). For example, seeds of *M. geometrizans* are described as oval by Barthlott and Hunt (2000); however, our results indicated they are almost round based on their roundness index value. In contrast, *S. queretaroensis* seeds were longer than wider. Moreover, Barthlott and Hunt (2000) mention that there is a high correlation between seed mass and size (length and width). In the studied species, the correlation coefficients were higher for mass vs. length than for width and length. Although *M. geometrizans* and *E. chiotilla* possessed the lighter and smaller seeds in the Pachycereeae studied, they are in the range of most Cactoideae seeds (Barthlott and Hunt, 2000). However, *P. grandis* surpassed the heaviest seeds reported for Cactoideae by these authors. Species differences just mentioned are consistent for each clade included in this study. Notably *E. chiotilla* and *M. geometrizans* belong to the same monophyletic clade sharing many of seed traits. Moreover, both species of *Neobuxbaumia* also showed the similar seed traits.

Germination

Most of the studied species germinated rapidly, as reported for other Cactaceae (Bregman and Bouman, 1983). None of the seeds died, and 100% of them germinated, mostly 18-24 days after sowing but never longer than 30 days. These results contrasted with other studies, where specific requirements are needed to improve germinability, for example seed scarification, different calcium concentrations, addition of gibberellic acid (GA_3), light treatments, and alternating temperatures (Nolasco *et al*., 1997, Godinez-Álvarez and Valiente-Banuet, 1998; Benítez-Rodríguez *et al*., 2004; Ramírez-Padilla and Valverde, 2005; Ortega-Baes and Rojas-Arechiga, 2007; Méndez, 2007). For the studied species, seed dispersal to appropriate safe sites for germination and seedling establishment is a critical process, which is affected by biotic and abiotic factors (Valiente *et al*., 1991; Reyes-Olivas *et al*., 2002), including size, morphology, and germinative response as it has been reported for diverse species of plants (Chambers and MacMahon, 1994). The smallest and lighter seeds of *E. chiotilla* and *M. geometrizans* showed a seed germination delay compared with the other species with larger and heavier seeds. Venable and Brown (1988) suggest that species with smaller seeds have more specific germinative requirements than the larger ones. An increment in seed size has an important effect on germination, as also occurred in seedling establishment, growth, and successful development of the cacti seedlings studied (Loza-Cornejo, 2004) as it occurs in other species (Fenner, 1992; Balogh and Toft, 2007).

In the studied species, the time of fruit ripening occurs at the end of the spring similar to other members of *Neobuxbamia, Pachycereus*, and *Stenocereus* (Dubrovsky, 1996; Pimienta-Barrios and Nobel, 1998; Flores and Briones, 2001). In contrast, fruit ripening for most species in *Opuntia* occurs from the middle of summer to the beginning of the autumn (Nobel, 1988; Mandujano *et al.,* 2005). Then columnar cacti seed maturation occurs just before the start of the summer, which favors seed germination and seedling establishment because of favorable soil humidity and shading by vegetation in their microhabitats.

We conclude that the 6 species studied have no dormancy as other requirements to successfully geminate just after fruits are harvested. Absence of dormancy for the studied seeds may be considered a selective advantage, given the short and unpredictable rainfall periods occurring in most arid and semiarid habitats (Pimienta-Barrios and Del Castillo, 2002). Moreover, our results supported the hypothesis that species from different monophyletic clades had different morphological seed traits, but relatively similar germinative response under laboratory conditions.

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Table 1. Geographic location and provenances of the six species studied, and Boucher specimen data.

Species	Geographic Location			Provenance
	N Latitude	W Longitude	Elevation (m asl)	
Escontria chiotilla	17°58’	99°32’	594	Xatitlán, Guerrero (Terrazas 407)
Myrtillocactus geometrizans	20°55’	99°46’	1560	Higuerillas, Querétaro (Terrazas 555)
Neobuxbaumia mezcalaensis	17°47’	99°33’	744	Cañón del Zopilote, Guerrero (Terrazas 404)
Neobuxbaumia multiareolata	17°10’	99°35’	450	Tierra Colorada, Guerrero (Terrazas 537)
Pachycereus grandis	18°13’	98°08’	1423	Acatlán, Puebla (Arias 1380)
Stenocereus queretaroensis	20°15’	103°34’	1360	Sayula, Jalisco (Arreola-Nava 1342)

Table 2. Mean (±sd) number of seeds per fruit, seed mass, length, width, and roundness index for 6 species of Pachycereeae.
Different letter in each column means significant difference ($P < 0.05$).

Species	Number	Mass (mg)	Length (mm)	Width (mm)	Roundness Index
Escontria chiotilla	410 ± 81^{d}	0.66 ± 0.10^{a}	1.7 ± 0.16^{a}	1.3 ± 0.14^{a}	0.77^{c}
Myrtillocactus geometrizans	122 ± 25^{b}	0.61 ± 0.18^{a}	1.5 ± 0.11^{a}	1.2 ± 0.08^{a}	0.81^{d}
Neobuxbaumia mezcalaensis	111 ± 51^{a}	8.25 ± 1.70^{c}	4.0 ± 0.29^{c}	3.1 ± 0.25^{c}	0.77^{c}
Neobuxbaumia multiareolata	160 ± 33^{c}	6.3 ± 0.97^{c}	3.6 ± 0.27^{c}	2.7 ± 0.23^{c}	0.76^{c}
Pachycereus grandis	597 ± 117^{e}	22 ± 3.60^{d}	5.3 ± 0.25^{d}	3.7 ± 0.24^{d}	0.70^{b}
Stenocereus queretaroensis	816 ± 237^{f}	2.27 ± 0.43^{b}	2.4 ± 0.16^{b}	1.6 ± 0.11^{b}	0.67^{a}

Table 3. Pearson correlation coefficients (r) and their significances among seed traits within each species (N = 300 for each species), ns = nonsignificant.

Species	Seed							
	Length vs. Width		Length vs. Mass		Width vs. Mass		Mass vs. Number	
	r	P	r	P	r	P	r	P
Escontria chiotilla	-0.12	0.04	0.09	ns	0.26	0.0001	-0.22	0.005
Myrtillocactus geometrizans	0.46	0.0001	-0.13	0.03	0.04	ns	0.05	ns
Neobuxbaumia mezcalaensis	0.32	0.0001	0.35	0.0001	0.33	0.0001	0.29	0.0001
Neobuxbaumia multiareolata	0.47	0.0001	0.09	ns	-0.01	ns	-0.14	0.01
Pachycereus grandis	0.52	0.0001	0.79	0.0001	0.70	0.0001	-0.05	ns
Stenocereus queretaroensis	0.71	0.0001	0.51	0.0001	0.46	0.0001	-0.10	ns

Table 4. Chi-squared values for log-rank paired comparisons between the studied species. In all cases d.f. = 1, n = 150 seeds, three replicates per species. (left superscript, w, for the chi-squared value indicates paired Wilcoxon test).

Species	*M. geometrizans*	*N. mezcalaensis*	*N. multiareolata*	*P. grandis*	*S. queretaroensis*
Escontria chiotilla	^{W}105.18***	79.02***	258.32***	26.52***	^{W}9.14**
Myrtillocactus geometrizans	—	171.54***	299.75***	167.08***	62.22***
Neobuxbaumia mezcalaensis		—	31.16***	^{W}72.63***	29.09***
Neobuxbaumia multiareolata			—	161.39***	108.56***
Pachycereus grandis				—	$^{W}0.061^{ns}$

* = $P < 0.01$; ** = 0.0025 *** = $P < 0.0001$; ns = nonsignificant

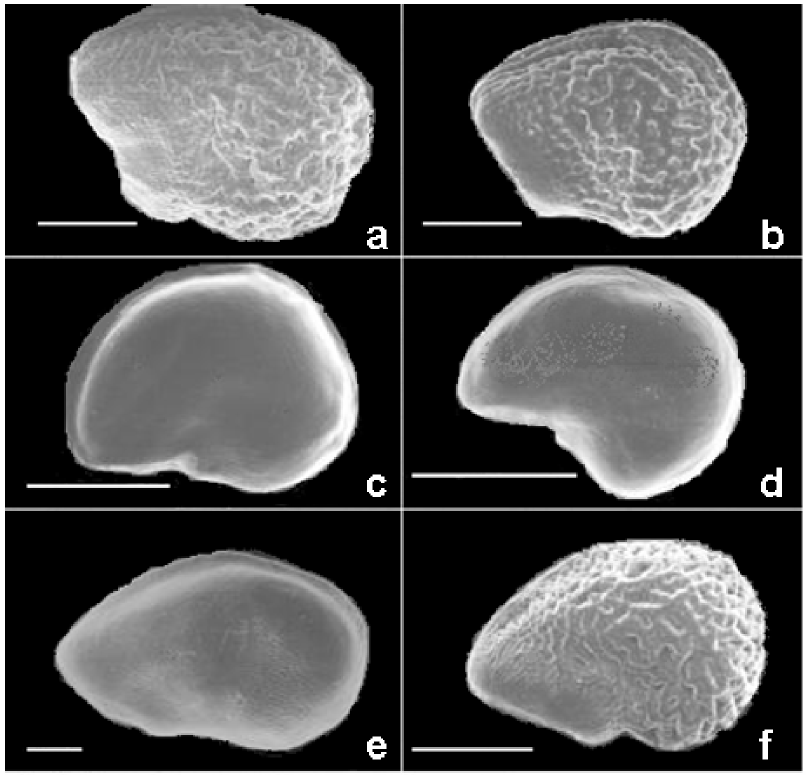

Figure 1. SEMs of Pachycereeae seeds. a. *Escontria chiotilla*. b. *Myrtillocactus geometrizans*. c. *Neobuxbaumia mezcalaensis*. d. *Neobuxbaumia multiareolata*. e. *Pachycereus grandis*. f. *Stenocereus queretaroensis*. Scale bar = 1 mm, e = 2 mm.

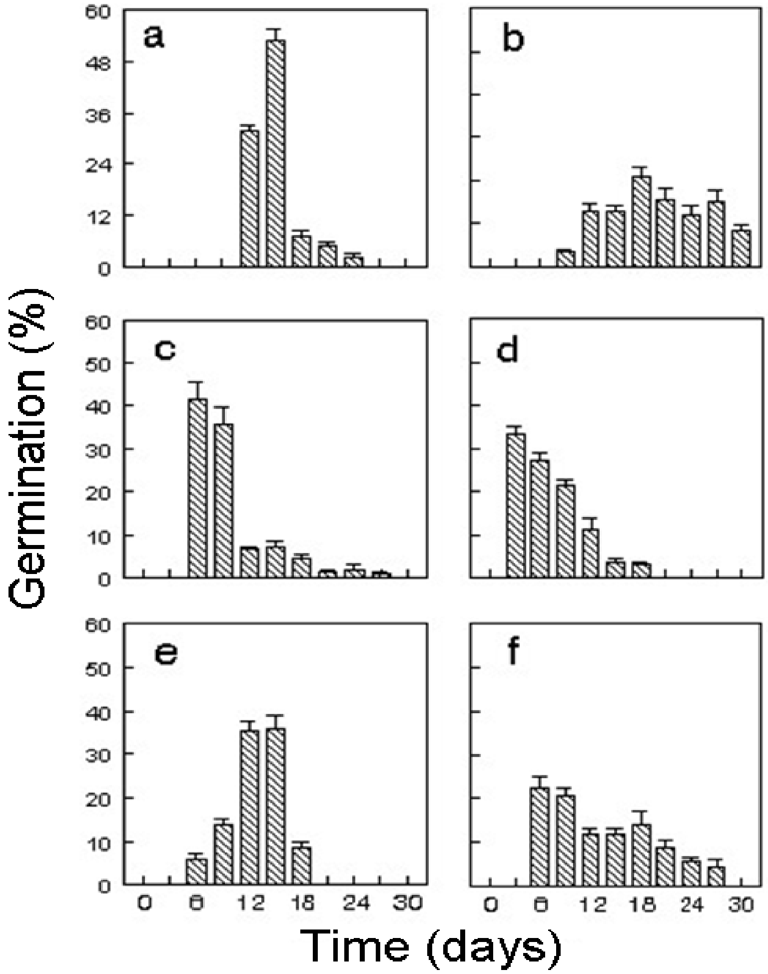

Figure 2. Seed germination percentage per day for six Pachycereeae species.
a. *Escontria chiotilla*. b. *Myrtillocactus geometrizans*. c. *Neobuxbaumia mezcalaensis*.
d. *Neobuxbaumia multiareolata*. e. *Pachycereus grandis*. f. *Stenocereus queretaroensis*.

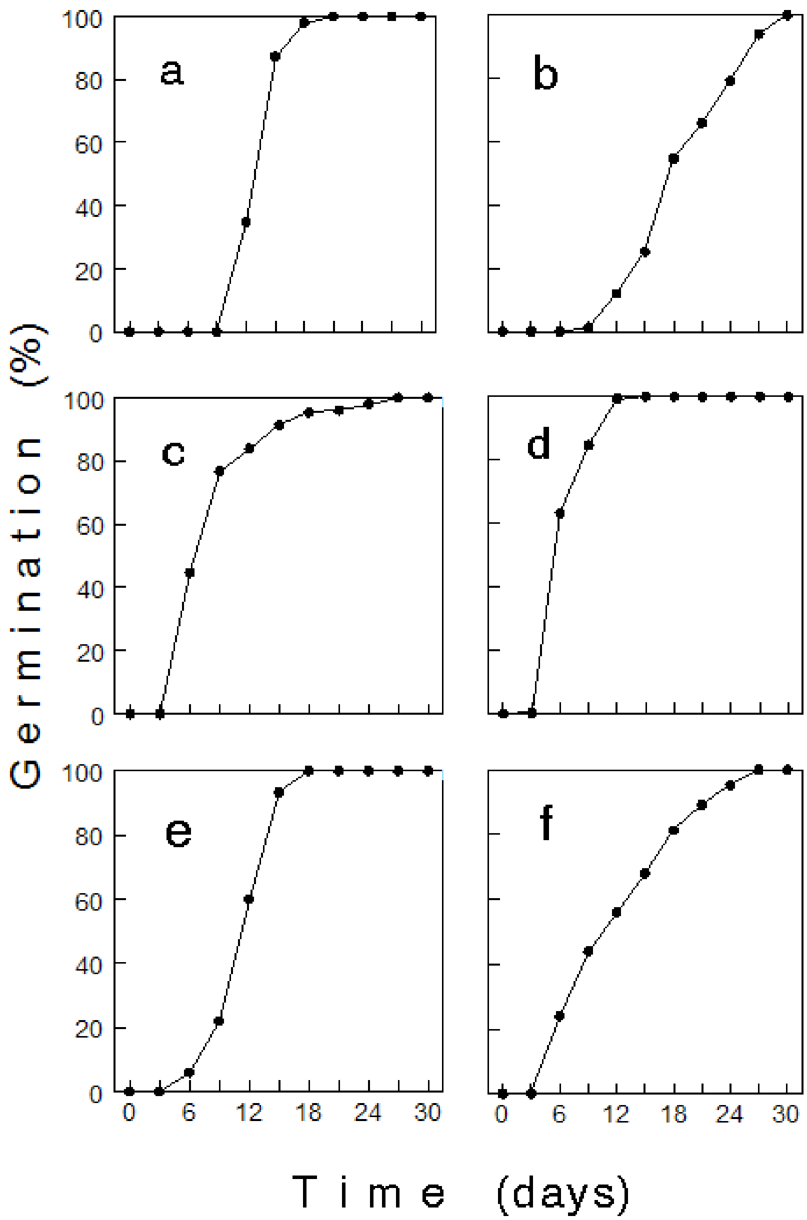

Figure 3. Figure 3. Patterns of accumulative seed germination for six Pachycereeae species.
a. ***Escontria chiotilla*.** b. ***Myrtillocactus geometrizans*.** c. ***Neobuxbaumia mezcalaensis*.**
d. ***Neobuxbaumia multiareolata*.** e. ***Pachycereus grandis*.** f. ***Stenocereus queretaroensis*.**