Heteromorphism in seeds of *Leptocereus scopulophilus* (Cactaceae) from Pan de Matanzas, Cuba

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Abstract

Seed heteromorphism is the formation of different seed morphs from the same individual. Two seed morphs have been preliminarily observed in *Leptocereus scopulophilus*. One morph shows an apparent natural scarification of its coat. Herein we describe the seeds, taking into account shape, coat integrity, surface, dimensions, mass and the position of germination cracks. We defined two seed morphs using the integrity of the spermoderma: fragmented seed coats (FSC) and complete seed coats (CSC). We also evaluated minimum germination time, germination rate and germinability. The seed morphs did not differ significantly in traits; however, regular striations along the cuticle of the periclinal walls were more visible in the FSC compared with the CSC. Both seed morphs displayed anticlinal cell boundaries in the border region that are channelled and straight in the dorsal-ventral region but difficult to define in the lateral region. We found four morphological variations in different positions where the radicle or cotyledons emerge and variations in cuticle thickness in different regions of the seed that could determine the formation of cracks during germination. All germination variants occurred in both seed morphs, albeit in different proportions. Germination was higher and faster for the FSC compared with the CSC. These germination differences could be related to a thinner cuticle in the FSC and the punctual release of its spermoderma, which facilitates a quick imbibition of the embryo and the breaking of the seed coat. Our results indicate that differences in germination parameters between the two seed morphs relate to differences in the percentage of dormant seeds, which favour the temporal expansion of germination and reduce competition between siblings. To propagate the species for conservation purposes, we recommend using FSC, while CSC may be used to establish a seed collection *ex situ*.

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Introduction

Seed germination and seedling establishment are critical stages in a plant's life cycle (Fenner, 1985; Pérez-Sánchez *et al.*, 2011; Arroyo-Cosultchi *et al.*, 2016). Therefore, the development of strategies that maximize survival throughout these phases are of vital importance for the perpetuation of plant species. One such strategy is seed heteromorphism (Matilla *et al.*, 2005), or the formation of different seed morphs from the same individual (Venable, 1985; Smith *et al.*, 2002). This strategy has been described for 200 species and 18 families of angiosperms, with *Asteraceae*, *Amaranthaceae* and *Brassicaceae* as the most represented (Matilla *et al.*, 2005).

Variations between seed morphs can be described in terms of size, shape, colour and seed mass, as well as seed-coat sculpturing, water permeability, cuticular structure, gene structure and embryo size (Smith *et al.*, 2002; Baskin and Baskin, 2014; Leverett and Jolls, 2014). Other variations between seed morphs also occur in seed behaviour without visible morphological differences (Venable, 1985). This type of seed heteromorphism, termed cryptic, has been observed in various species (Leverett and Jolls, 2014; Liyanage *et al.*, 2016), and is a common, yet rarely documented phenomenon (Venable, 1985; Leverett and Jolls, 2014). The ecological advantages of seed heteromorphism include differences between morphs in competitive ability, dispersion, dormancy, germination time, vulnerability to predators and seedling growth (Matilla *et al.*, 2005; Lu *et al.*, 2010; Leverett and Jolls, 2014). However, understanding the selective forces that favour the evolution and maintenance of seed heteromorphism through evolutionary time remains a key question in studies dealing with heteromorphism; these forces probably depend on habitat and the mechanisms controlling population dynamics in the communities in which heteromorphism occurs (Liyanage *et al.*, 2016).

In the field we observed two seed morphs in *Leptocereus scopulophilus* Areces, an endemic threatened Cuban cactus. One of these morphs shows an apparently natural scarification of the seed coat, which may indicate a zone of lower mechanical strength favourable for germination. Seed heteromorphism in Cactaceae is barely documented. It has been reported for both seed size and mass in *Astrophytum myriostigma* Lem. (Sánchez-Salas *et al.*, 2006) and *Stenocereus beneckei* (Engelm.) Buxb. (Ayala-Cordero *et al.*, 2004). In these species, seed size implies differences in germination capacity and speed (Ayala-Cordero *et al.*, 2004; Sánchez-Salas *et al.*, 2006). However, to date no study on seed heteromorphism has reported apparently scarified seeds, and as such the possible implications of this trait in the germination of the species that possess it are not known.

In this study, the two seed morphs of *L. scopulophilus* are characterized for the first time; germination is described for each morph and performance is compared between the two morphs. We hypothesize that there are differences in germination capacity between the two seed morphs. Moreover, this study helps to clarify the implications of seed heteromorphism in the germination process of this cactus, which provide essential elements to consider in the conservation strategies of *L. scopulophilus*.

Materials and methods

Study area

Leptocereus scopulophilus occupies 3500 m^2 of the deep southeastern slope of Pan de Matanzas, a karstic hill located 11 km west the city of Matanzas, Cuba. The population occurs at 212 m above sea level, in the understory of a semi-deciduous tropical forest (Barrios *et al.*, 2012). The Pan de Matanzas hill has a seasonal tropical climate with a dry season in the winter. The dry season lasts from November to April with an average rainfall of 331 mm and an average temperature of 23.6°C. The rainy season begins abruptly by May until October with an average rainfall of 1066 mm and an average temperature of 25.8°C. Relative humidity varies from an average of 82.0% during the dry season to 89.4% in the rainy season. Predominant soils are shallow and rocky, belonging to the calcimorphic humic group: red rendzinas, black rendzinas and humic-carbonated (Barrios *et al.*, 2015).

Species

Leptocereus scopulophilus is a critically endangered columnar tree-like cactus, endemic to the karstic elevations of Mayabeque and Matanzas (Barrios, 2014).

The fruits are ellipsoid-globose, 6.5 cm long and 5.5 cm wide, and yellowish-green coloured with persistent areolas at maturity (Areces, 1993). Seeds are dark and small, with dimensions reported for the population of Loma de Somorostro of 1.3 mm long and 0.9 mm wide (Areces, 2003). Moreover, all seeds are surrounded by mucilage with a pectin matrix (Barrios *et al.*, 2015). The seeds are dispersed by an endemic rodent, *Capromys pilorides* (Barrios *et al.*, 2012).

Morphological analysis of seeds

In November 2013, ten mature fruits were randomly collected from ten different individuals and 50 seeds of each morph were selected (five seeds of each morph per fruit). The fruit was cut longitudinally spanning only the pericarp (so as to not damage the seeds); both parts of the fruit were rotated in opposite directions until split, and the pulp containing the seeds was removed using a spoon. We considered a mature fruit a light green, turgid fruit with areoles spaced far from one another. Morphs were differentiated using the presence or absence of spermoderma scarification. When describing the seeds, we noted qualitative characteristics such as shape and coat integrity, as well as quantitative characteristics such as length, width, length of the hilum-micropylar region, thickness (Fig. 1) and mass. The measurements were made with a caliper (±0.05 mm) and an analytic Sartorius balance (±0.1 mg).

In order to identify morphological differences between the two seed morphs, the seed surface was studied by electron microscopy equipped with Hitachi S-405A and CamScan-S2 after being coated with gold–palladium in an argonic medium. Photomicrographic analysis used the following sequence of description: (1) spermoderma cell shape, (2) anticlinal cell boundaries (relief, curvature and interstices), and (3) periclinal wall sculpture (curvature and surface). The terminology follows the criteria of Barthlott and Hunt (2000).

Germination of seed morphs

Two hundred and forty seeds of each morph were collected from eight fruits (30 seeds per fruit for each morph) from eight different individuals. The seeds were selected and washed with running water until the mucilage was removed, as it can delay seed germination of *L. scopulophilus* (Barrios *et al.*, 2015). A paired experiment with eight replicates of 30 seeds per morph were sown in Petri dishes (10 cm diameter and 1 cm depth), with two layers of filter paper moistened with distilled water used as a substrate. This experiment was performed in nursery conditions
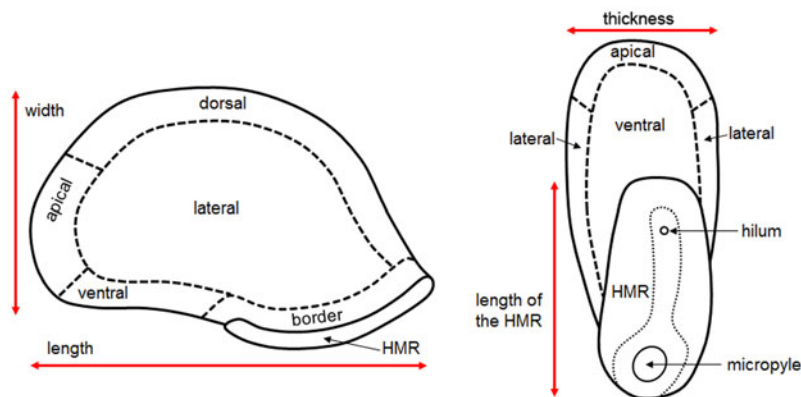
Figure 1. Traits analysed and regions of *Leptocereus scopulophilus* seeds. A, side view; B, ventral view. HMR: hilum-micropylar region.

under mesh-filtered light (3515 lx). Day length (irradiation time per day) was 11–12 h and temperature was an average of 24.3°C with a maximum of 32.6°C and a minimum of 20.5°C. These conditions are similar to those recorded during *L. scopulophilus* germination experiments by Barrios *et al.* (2015). Both light and temperature were recorded every 30 min using a data logger (Hobo) (±0.1 lx and ± 0.1°C) during the experiment. To prevent fungal growth, six drops of 1% sodium hypochlorite were added at the beginning of the experiment according to Naranjo *et al.* (2003). Distilled water was added to the saturation point weekly using a sprayer to maintain moisture. For each germinated seed the following features were determined: (1) the seed region from where the embryo emerged (Fig. 1), (2) the primary organ emerged (radicle or cotyledons), (3) the position of the germination cracks, and (4) operculum formation.

The germination behaviour of these two morphs was evaluated by three parameters: (1) minimum germination time (T_{min}), which is the minimum time required for the seeds to start germination; (2) germination rate (T_{50}), which indicates the time necessary for half of the seeds to germinate relative to the total germinating seeds; and (3) germinability (*G*) in 28 days, which refers to the percentage of seeds germinated under experimental conditions (Flores and Briones, 2001). Visible emergence of the cotyledons or the radicle through the spermoderma was defined as germination. T_{50} was calculated according to Ranal *et al.* (2009) as:

$$T_{50} = \frac{\Sigma n_i \cdot t_i}{\Sigma n_i}$$

where n_i is the number of seeds germinated at time *i* and t_i is the incubation period in days.

Data analysis

For all variables analysed, normality of the data was checked using a Kolmogorov–Smirnov and Levene test for homogeneity of variance. Seed traits T_{min} and T_{50} were each compared between seed morphs using a *t*-test. Germinability and the frequency of germination variants were compared with binary logistic regression (Sileshi, 2012). All statistical analyses were performed using the STATISTICA version 10.0 program.

Results

Seed morphs

Leptocereus scopulophilus seeds are oval, mussel-shaped and flattened laterally. We define two seed morphs via the integrity of the spermoderma: (1) fragmented seed coats (FSC), which appear scarified (Fig. 2) due to a punctual release of the coat, usually towards the apical tip in both dorsal–ventral and lateral regions (Fig. 3A), and (2) complete seed coats (CSC) (Figs. 2 and 3B). The seed morphs did not differ significantly in traits (Table 1).

For both morphs, spermoderma cells are isodiametric and quadrangular to hexagonal on the border and lateral regions. Spermoderma cells in the dorsal–ventral region, however, are elongated. In this region, the relief of the anticlinal boundaries are channelled and straight, with pronounced craters at the intersections between cells and a convex curvature of the periclinal wall with irregular parallel striations along the cuticle (Fig. 3C). Anticlinal cell boundaries in the border region were variable in both seed morphs. These cell boundaries may either be raised or inconspicuous, in this case with pronounced craters at the intersections
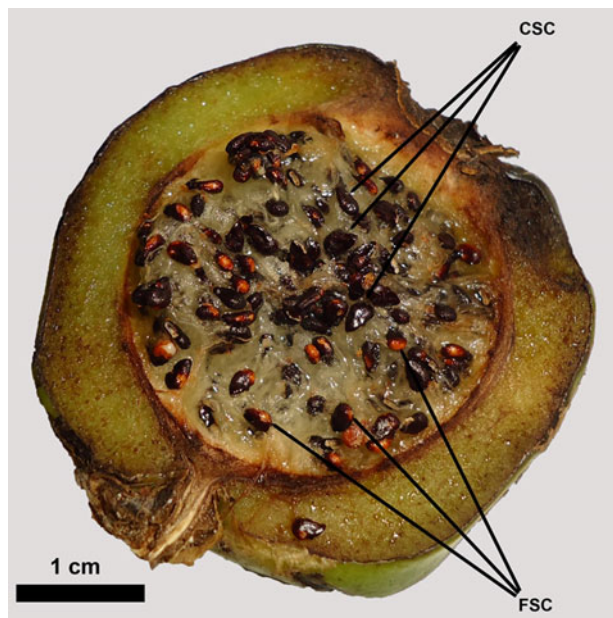
Figure 2. Longitudinal section of *Leptocereus scopulophilus* fruit showing fragmented seeds coat (FSC) and complete seed coats (CSC).

between cells. In the lateral region, anticlinal cell boundaries are very hard to define (Fig. 3D and E). Additionally, irregular striations along the cuticle of periclinal walls were more visible in the FSC compared with the CSC (Fig. 4).

Germination of seed morphs

Four morphological variations were observed where the radicle or cotyledons emerge from the germination cracks in different positions on the spermoderma (Fig. 5). The most frequent variant was operculed hilum-micropylar germination in CSC, which had a different frequency in FSC (Table 2). Operculed hilum-micropylar germination was characterized by the formation of a large dorsal crack (covering 3/4 of the dorsal region of the seed) and two lateral cracks originating near the dorsal edge of the hilum-micropylar region extending to the ventral side of the seed (Fig. 5A). As a result, the hilum-micropylar regions, together with one or more rows of cells in the border region, constitute the operculum, which is pushed during the emergence of the radicle.

Dorsal unoperculed germination (Fig. 5B) occurs due to the formation of a large dorsal crack (covering the entire dorsal region of the seed), which is followed by a single lateral crack that originates near the dorsal edge of the hilum-micropylar region and extends to the ventral side of the seed. Thus two valves originate from the seed coats, where the radicle emerges. There is no operculum, as one of the lateral cracks is not formed,

and the hilum-micropylar region is attached to one of the valves. In dorsal unoperculed germination, similar to the operculed hilum-micropylar variant, the cracks occur both on the seed coat and on the thin endosperm layer.

Other germination variants were labelled as alternative due to the emergence of the embryo in an area opposite to the point of emergence in previous variants. Alternative unoperculed germination (Fig. 5C) is almost exclusive to FSC (Table 2) and a short crack is formed where the inner integument is exposed at the apical end of the seed. In this germination variant the endosperm and the membrane formed by the inner integument break off and the cotyledons appear through the crack. Finally, in alternative operculed germination (Fig. 5D), a transverse crack is formed at the dorsal region and towards the apical end of the seed, which is continued on both sides until the opposite point of the transverse cracks. As a result, a circumcised apical operculum is formed through which the cotyledons appear.

All germination variants were found in both seed morphs. However, in the CSC, operculed hilum-micropylar germination predominates, while in the FSC, operculed hilum-micropylar germination and dorsal unoperculed germination occurred in similar proportions (Table 2). Moreover, there was a better representation of the alternative germination variants in FSC compared with CSC (Table 2). The minimum germination time (T_{min}) and germination rate (T_{50}) of the FSC were significantly lower and the germinability (G) significantly higher compared with the CSC (Table 3). Germination was delayed in the CSC and took almost twice as long as in FSC, and the germination rate in FSC was increased by 7 days relative to CSC (Table 3).

Discussion

Characterization of seed morphs

The traits of *L. scopulophilus* seeds did not significantly vary between morphs. However, it is remarkable that the seeds collected in the population of the Pan de Matanzas hill are double the size in length and width of those reported by Areces (2003) from the Somorrostro Hill population. The results from Areces (2003) may be biased due to the seeds originating from a single individual collected in 1923. However, seed size might be a character of geographic variation in *L. scopulophilus*, which has been shown to influence the survival of seedlings in several species (Venable, 1992; Andersson and Milberg, 1998).

On the other hand, the elongated shape of the coat cells of the dorsal–ventral region agree with Areces (2003), who suggests that these cells in *L. scopulophilus*
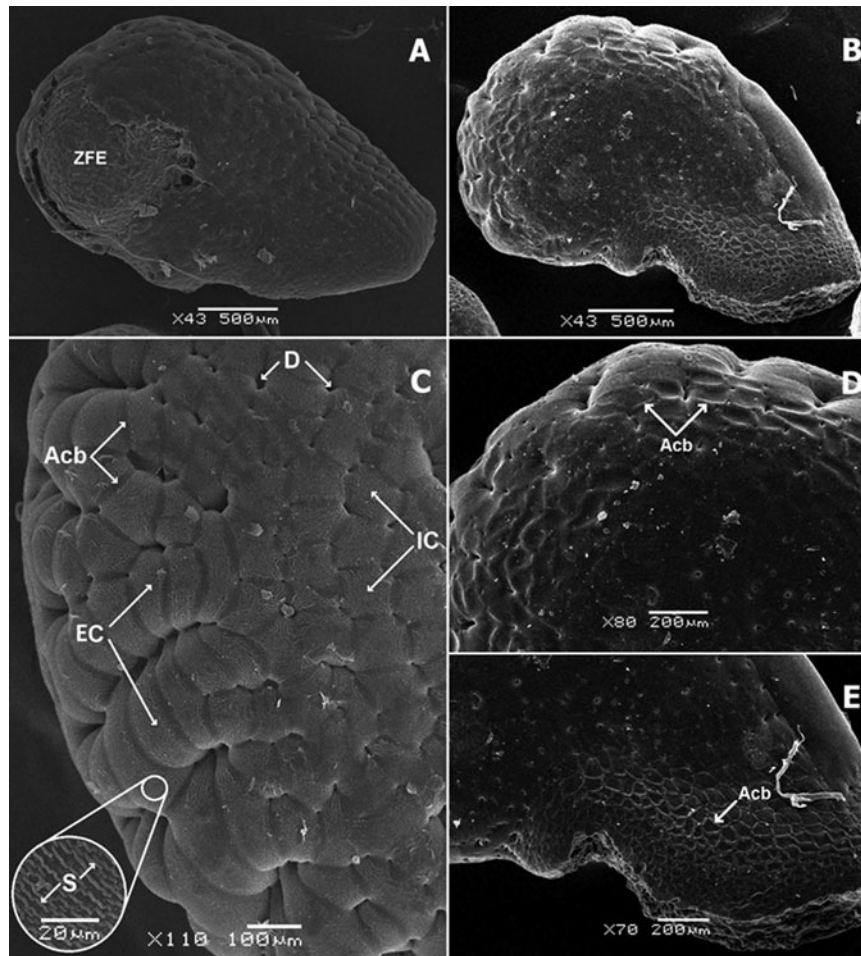
Figure 3. *Leptocereus scopulophilus* seeds. A, fragmented seed coat at zone with fragmented spermoderma (ZFE) in the apical end of the dorsal-ventral and lateral regions; B, complete seed coat; C, dorsal-side view of spermoderma surface in fragmented seed coat, anticlinal cell boundaries (Acb), depression (D), isodiametric cells of the lateral region (IC), elongated cells in the dorsal–ventral region (EC) and striations (S); D, complete seed coat with conspicuous anticlinal cell boundaries (Acb) in the dorsal–apical region and E: in the border region.

are the only exception to the isodiametric pattern that characterizes the spermoderma cells of *Leptocereus*. Moreover, the visibility of anticlinal walls, and the irregular striations along periclinal walls, provide evidence for the existence of a thin cuticle in *L. scopulophilus* seeds. This result agrees with Areces (2003), who includes the species in a group with a fine or regular

cuticle. We consider that the presence of irregular striations along periclinal walls of the dorsal region in FSC is an indicator of a thinner cuticle in this morph relative to CSC.

Germination of seed morphs

Two seed morphs in *L. scopulophilus* with different germination behaviour may represent a survival mechanism in the face of environmental variability, as has been suggested by Venable (1985) and Lu *et al.* (2014). This mechanism may act by spreading germination and seedling recruitment over a longer period of time (Lu *et al.*, 2010; Leverett and Jolls, 2014), thus reducing competition between siblings (Cheplick, 1996) and seedling predation (Janzen, 1970; Schupp, 1988). Currently, seed dispersal of *L. scopulophilus* covers very short distances, usually under the mother plant, while the germination of its seeds occurs mainly

Table 1. Seed traits of fragmented seeds coat (FSC) and complete seeds coat (CSC) in *Leptocereus scopulophilus* (*n* = 50)

Seed trait	FSC (mean ± SE)	CSC (mean ± SE)	*t*	*P*
Length (mm)	2.76 ± 0.05	2.84 ± 0.05	−1.03	0.318
Width (mm)	1.65 ± 0.03	1.70 ± 0.03	−0.86	0.402
HMR (mm)	1.73 ± 0.02	1.81 ± 0.03	−1.76	0.096
Thickness (mm)	1.15 ± 0.04	1.10 ± 0.01	0.98	0.338
Mass (mg)	2.08 ± 0.13	2.10 ± 0.13	−0.11	0.916

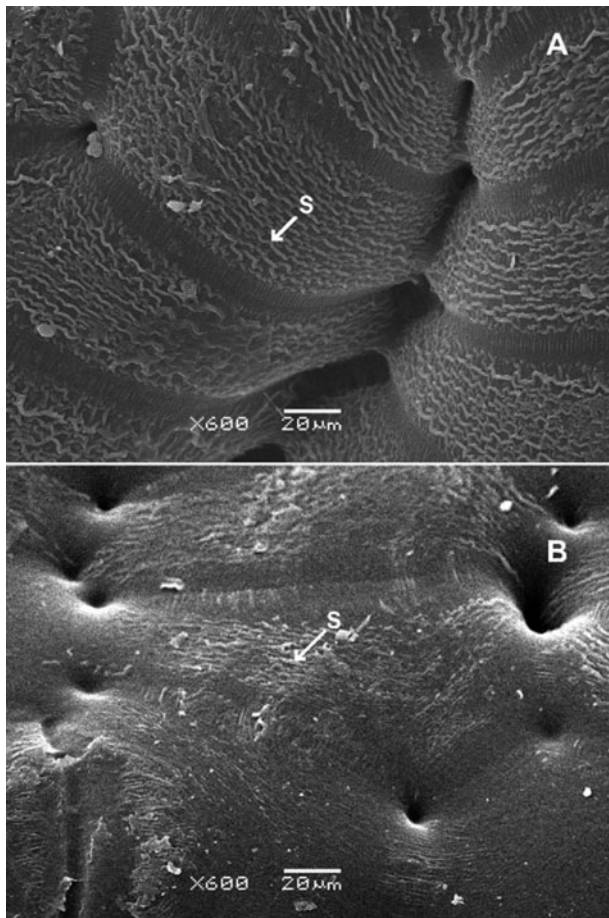HMR: length of the hilum micropylar region.

Figure 4. Striations (S) of the periclinal wall cuticle of a fragmented seed coat (A) and a complete seed coat (B) in *Leptocereus scopulophilus*. Dorsal views.

Figure 5. Germination variants in *Leptocereus scopulophilus*. Continuous lines indicate the position of germination cracks occurring in spermoderma. Dashed lines indicate the position of germination cracks on the opposite seed side. A, operculed hilum-micropylar germination; B, dorsal unoperculed germination; C, alternative unoperculed germination; D, alternative operculed germination.

in the rainy season. Given this behaviour, the successful survival of the seedlings of this endemic cactus will depend on the balance between rapid germination (to accumulate sufficient water during the rainy season) and avoiding herbivory, which may be intense at the start of the rainy season and reduced during the dry season (Gonzalez-Torres *et al.*, 2012). Otherwise, having two different germination behaviours may ensure that the population persists in case of seedling establishment failure at a given moment during the rainy season (Venable, 1985). In this way, seed heteromorphism in this cactus may act as a response to the environmental variability (temperature, rain patterns, relative humidity and sunlight) to which the population of Pan de Mantanzas is subjected (Gonzalez-Torres *et al.*, 2012).

Four germination variants were observed in the present study for *L. scopulophilus* seeds, which contrasts with what was reported for the genus by Areces (2003). This author studied the germination of only two species of *Leptocereus* (*L. quadricostatus* and *L. sylvestris*) and their work was fundamentally guided by taxonomic questions, leading them to recognize only the
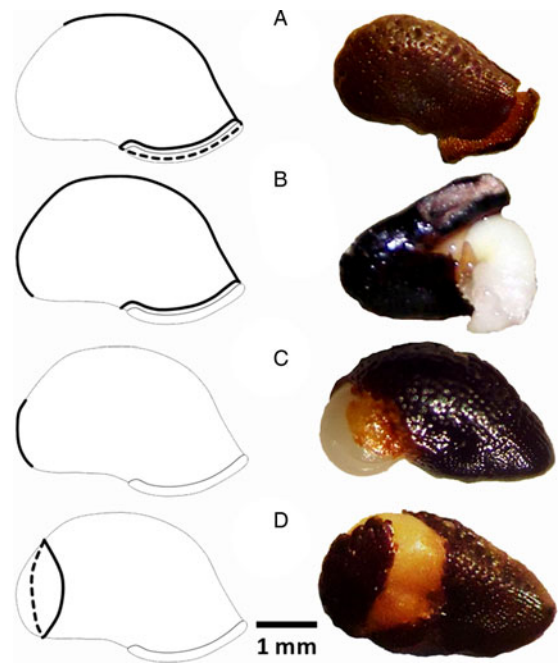
operculed hilum-micropylar germination, which corresponds to the '*Cereus*' variant described by Bregman and Bouman (1983). A thinner cuticle on the border region could contribute to this variant, since the lateral cracks that release off the operculum (hilum-micropylar region) occur in the basal region according to Bregman and Bouman (1983) and Areces (2003). The '*Cereus*' variant is the most widespread in the family Cactaceae. According to Vishenskaya (1991), this seed variant is favoured ecologically due to the ability to rapidly germinate in a short period of rain. However, the dorsal unoperculed germination variant represents more than 30% of variants in each seed morph of *L. scopulophilus*, and it is very similar to the *Pereskia* variant described by Bregman and Bouman (1983). Therefore the '*Cereus*' variant might be derived directly from the *Pereskia* variant and not indirectly as argued by Bregman and Bouman (1983). Moreover, another two germination variants in *L. scopulophilus* are treated as exceptions by Bregman and Bouman (1983). According to these authors, exceptional variations often occur in seeds of the '*Cereus*' variant because the lateral cracks that form the hilum-micropylar operculum are not formed. The alternative germination variants occur only if one apical crack is formed prior to the formation of the dorsal crack which initiates the predominant variants. This could be related to the lower mechanical strength at the apical region of the

Table 2. Germination variants of fragmented seeds coat (FSC) and complete seeds coat (CSC) in *Leptocereus scopulophilus*

Germination variants	FSC (% ± SE)	CSC (% ± SE)	χ^2	P
Operculed hilum-micropylar	34.50 ± 4.16	63.40 ± 8.81	6.97	**0.008**
Dorsal unoperculed	34.90 ± 3.24	32.30 ± 6.59	0.33	0.560
Alternative unoperculed	21.71 ± 5.17	1.24 ± 1.28	18.89	**0.0001**
Alternative operculed	8.93 ± 2.25	3.11 ± 2.57	1.86	0.172

$n = 8$; P-values in bold are statistically significant (at the 5% level)

Table 3. Germination of fragmented seeds coat (FSC) and complete seeds coat (CSC) in *Leptocereus scopulophilus*

Parameters	FSC (mean ± SE)	CSC (mean ± SE)	Test	P
T_{min} (days)	6.5 ± 0.23	12.12 ± 1.34	–4.11	**0.0010**
T_{50} (days)	11.37 ± 0.46	18. 87 ± 1.48	–4.83	**0.0002**
G (%)	96.25 ± 1.93	67.08 ± 8.80	11.80	**0.0006**

$n = 8$; Test is t-test for T_{min} and T_{50}, χ^2 for G; P-values in bold are statistically significant (at the 5% level). T_{min}, minimum germination time; T_{50}, germination rate; G, germinability.

FSC, the seed morph where these alternative variants are well represented. Our results show that the germination variant determines the primary organ of the embryo that is going to emerge. In alternative variants cotyledons emerge first, while in the predominant variants it is the radicle which emerges primarily, as has also been reported by Bregman and Bouman (1983). This is due to the curved shape of the embryo in the Cactaceae family, where the apical end of the seed corresponds to the cotyledons and the border region to the radicle (Bravo-Hollis and Sanchez-Mejorada, 1991).

High and fast germination of the FSC compared with CSC provides evidence for physiological differences in germination, as mentioned by Matilla *et al.* (2005) for species with seed heteromorphism. Germination differences between the morphs could be related to a thinner cuticle in the FSC and the punctual release of its spermoderma, which facilitates a quick imbibition of the embryo and the coat breaking. The relationship between spermoderma characteristics and germinability for FSC agrees with the idea proposed by Maiti *et al.* (1994), where a high percentage and quick germination of seeds in *Cactaceae* may be related to the presence of a thin spermoderma. A similar observation has been reported for *Phaseolus vulgaris* L. (Powell *et al.*, 1986), *Brassica rapa* L. (Puga-Hermida *et al.*, 2003) and *Arabidopsis thaliana* (L.) Heynh. (Kuang *et al.*, 1996). In these species, the seed morphs with thinner spermoderma have a higher rate of imbibition and an increased percentage of germination (Matilla *et al.*, 2005). Moreover, the FSC may originate from changes in development that cause loosening of the spermoderma in certain parts of the coat along with decreased pigmentation. Thus FSC have adaptive advantages in the use of water in drier substrates and may influence the occurrence of vivipary in the seeds of the inner fruit of *L. scopulophilus*, as reported by Barrios *et al.* (2012). However, until now, no studies of vivipary in Cactaceae have reported seed heteromorphism or FSC (Conde, 1975; Cota-Sánchez, 2004; Cota-Sánchez & Abreu, 2007; Cota-Sanchéz *et al.*, 2007, 2011; Rojas-Aréchiga and Mandujano-Sánchez, 2009; Aragón-Gastelum *et al.*, 2013). Given this evidence, it is not possible to identify the punctual release of the FSC coat as the triggering factor for vivipary in the species. While we have observed that in *L. scopulophilus* the viviparous seedlings arise from FSC most frequently (probably as a consequence of the majority representation of FSC in alternative variants of germination; see Table 2), we have also found viviparous seeds in CSC, as shown in Fig. 1 of Barrios *et al.* (2012).

Having a complete spermoderma with a thicker cuticle also has its advantages. This characteristic of CSC can be a barrier to pathogens and increase the resistance to degradation during passage of the seed through the digestive tract of the dispersers. It may also allow for greater seed persistence in the soil, as suggested by Matilla *et al.* (2005). While in the present study we did not confirm the viability of the seeds, it is very likely that the 33% of the CSC which did not germinate in 4 weeks are dormant seeds, a trait that is related to the formation of a seed bank (Álvarez-Espino *et al.*, 2014). *Leptocereus scopulophilus* possesses permeable seeds that imbibe 25% of their fresh mass in the first 24 h; additionally, tests of viability conducted in our laboratory (both tetrazolium tests and cut tests) demonstrated that the seeds of *L. scopulophilus* that did not germinate for 4 weeks showed more than 90% viability (authors' unpublished data). Moreover, Barrios *et al.* (2015), under similar conditions to those in the present study, observed 3–21% germination of seeds of *L. scopulophilus* after 4 weeks, which can be considered dormant according to the criteria proposed by Baskin and Baskin (2014). Furthermore, the presence of fruit in the species throughout the entire year (Gonzalez-Torres *et al.*, 2012) may be further evidence that its seeds reach the ground at different

times, and at least one transient seed bank is expected. Seal *et al.* (2009) published a database which included 83 cactus species with seeds still viable a year after collection and 92 species with seeds tolerant to desiccation. Considering the results of this compilation, as well as the work of Barthlott and Hunt (2000) and Rojas-Aréchiga *et al.* (2013), it can be inferred that the majority of cactaceous seeds that have been studied show appropriate characteristics (small seeds with low mass and positive photoblastism for the persistence of seed banks in soil) (Baskin and Baskin, 2014). Although Rojas-Aréchiga and Batis (2001) mention that the formation of seed banks in cacti is unlikely due to the strong predation of seeds, some authors have noted its existence in *Echinopsis atacamensis* subsp. *pasacana* (de Viana, 1999), *Ferocactus wislizenii* (Engelm.) Britton & Rose, *Mammillaria grahamii* Engelm. (Bowers, 2000, 2005), *Opuntia engelmannii* Salm-Dyck (Montiel and Montaña, 2003), *Ferocactus viridescens* (Torrey & Gray) Britton & Rose (Angoa-Román *et al.*, 2005), *Harrisia fragrans* Small *ex* Britton & Rose (Goodman *et al.*, 2012), *Stenocereus stellatus* (Pfeiff.) Riccob. (Álvarez-Espino *et al.*, 2014), *Cereus jamacaru* DC. (Santos *et al.*, 2015) and in eleven more cacti species of Zapotitlán (Cano-Salgado *et al.*, 2012). The possible formation of at least one transient seed bank in the soil by CSC of *L. scopulophilus* would be an effective strategy for survival during monthly episodes of drought.

Leptocereus scopulophilus is a critically endangered species (González-Torres *et al.*, 2016) with two populations extending no more than a hectare, even though their areas of distribution were probably larger in the past (Barrios, 2014). In accordance with our results we propose the use of FSC for the propagation of the species, given its rapid and high germination capacity. The propagation of the species and its maintenance could be costly, both in terms of introducing it into nature and maintaining it in living collections in botanical gardens. On the other hand, according to Roberts (1991), establishing a seed collection *ex situ* would be a more efficient and less costly conservation method, in addition to providing a greater genetic representation of the population when compared with maintaining living collections (Cochrane *et al.*, 2007). We believe that the use of CSC would be more appropriate in establishing a seed collection *ex situ*.

In general, seed heteromorphism in *L. scopulophilus* may ensure successful germination and seedling establishment when considering variable microclimatic conditions and dispersion patterns. In further studies, it would be appropriate to evaluate the seed germination and seedling establishment of both morphs under different conditions (e.g. different water stress and temperatures) as well as the duration of seed viability. This will further clarify the contribution of both morphs to the reproductive success of *L. scopulophilus*.

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