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Article title: **Are seed mass and seedling size and shape related to altitude? Evidence in *Gymnocalycium monvillei* (Lem.) Britton & Rose (Cactaceae)**

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Abstract: Several studies reported a negative relationship between altitude and seed mass. In cacti species, seed mass has been also related to seedling morphology (size and shape). Here we studied *Gymnocalycium monvillei* (Lem.) Britton & Rose, a cactus species with a wide altitudinal distribution, with the main aim of analyzing how altitude affects seed mass and seedling size (height and width) and shape (globose or columnar seedlings). We collected seeds from five sites along the entire altitudinal distribution of the species in Córdoba Mountains (sites were located between 878 and 2230 m a.s.l.), encompassing a marked climatic gradient (6 °C of mean annual temperature difference between the extreme sites). Seed mass and seedling traits were measured in the laboratory. Seedling height increased with altitude, whereas seed mass was not related to this parameter. Seedlings became more globose (reduced surface/volume ratio) with decreasing altitude. Variation in seedling shape along the altitudinal gradient may be related to the contrasting climatic conditions to which seedlings are exposed, and could account for the wide altitudinal distribution of *G. monvillei*. Our results highlight the importance of seedling traits in the species response to climatic change.

Key words: Altitudinal gradients, *Gymnocalycium,* Seed mass, Seedling morphology, Cactaceae

Introduction

Seed size has been related to both latitudinal and altitudinal gradients. Baker (1972) and Murray et al. (2003) found that seed size decreases with increasing altitude, whereas Moles et al. (2007) and Murray et al. (2003) found that seed size decreases with increasing latitude. These patterns have been attributed to the increasing severity of environmental conditions as temperature decreases, reduced amount of photosynthates available to seeds, or simply to changes in plant growth forms along the gradients. However, how this pattern is maintained at the intraspecific level is still not clear (Pluess et al. 2005; Guo et al. 2010). In addition, to our knowledge, no studies have analyzed inter- or intraspecific changes in seed size along altitudinal gradients in cactus species.

Seedling size is an important character determining plant survival and growth (Leishman et al. 2000; Walters and Reich 2000), and depends strongly on seed size (Wulff 1986; Moles and Westoby 2004). In a study involving 17 cacti species, Sosa Pivatto et al. (2014) found that seed size was related not only to seedling size, but also to seedling shape: species with bigger seeds produced bigger seedlings, which were also more columnar (as adults, cactus seedlings are succulent). The authors stated that columnar seedlings would be better competitors for light, but globose seedlings would be more resistant to higher temperatures or drought events due to their lower surface/volume ratio (Felger and Lowe 1967; Cornejo and Simpson 1997). Low surface/volume ratio means that cacti have a small photosynthetic surface compared to the proportionally large respiratory demand of their water-storing parenchyma, and are therefore restricted by the amount of photosynthetically active radiation that is intercepted by the plants (Nobel 2003; Martorell et al. 2006).

Gymnocalycium monvillei (Lem.) Britton & Rose is a globose cactus endemic to Córdoba Mountains, central Argentina, and presents a very wide altitudinal distribution (Charles 2009; Gurvich et al. 2014). This gradient is related to important differences in temperature and humidity, which generate very different environmental conditions that could affect germination and establishment (García-Pérez et al. 2007). Studies of regenerative traits along gradients may help to understand how species cope with environmental conditions, and are necessary to predict the effect of climatic change on species abundances and distribution (Butler et al. 2012; Dávila et al. 2013). The aim of this study was to analyze how altitude affects seed size, and seedling size and shape (columnar or globose seedlings) of the cactus species*G. monvillei* by comparing different populations along its entire altitudinal distribution.

Materials and Methods

Study species, area characteristics and measured variables

Gymnocalycium monvillei inhabits rocky outcrops from 880 to 2200 m a.s.l. (Demaio et al. 2011; Gurvich et al. 2014). We studied five populations located along an altitudinal gradient between the localities of Cuesta Blanca (31° 28´ S, 64° 34 W) and Pampa the Achala (31° 41´ S, 64° 50´ W), at 878, 1250, 1555, 1940 and 2230 m a.s.l. (Fig. 1), each population corresponding to an altitudinal class. Mean annual temperature and precipitation varied from 16.5 °C and 680mm, to 10.3 °C and 790 mm, respectively, between the lowest and highest sites of this altitudinal gradient (de Fina 1992). Vegetation varied from subtropical dry forest to cold-temperate grasslands at the extreme sites (Giorgis et al. 2011). Frost can occur all year round and snow events are common during winter at sites above 1900 m a.s.l.

Mature fruits from 20 individuals were collected from each altitudinal class. Fruits were collected from individuals of similar sizes (around 10cm of diameter) for all altitudinal classes. Seeds were air-dried and stored in the laboratory at ambient conditions until the start of the experiments. We estimated seed mass by determining the weight of 250 dry seeds per population with a precision balance (0.1 mg). Seeds were weighed in 25 groups of 10 seeds because of their low weight.

Seeds were germinated in Petri dishes in a germination chamber under controlled light (12/12 h daily photoperiod of about 38 µmol m^{-2} s^{-1} cool white fluorescent light tubes) and temperature (25 °C) conditions. Thirty days after germination (considered as radicle protrusion), seven seedlings per altitude class were measured, following the procedure provided in Sosa Pivatto et al. (2014). Digital photographs were taken for each seedling, and seedling height and width were measured using BIO7 software. With these data we calculated a shape index (height/width ratio), with a value of 1 indicating spherical–globose– seedlings, and a value departing from 1 indicating that seedlings become cylindrical –the typical shape of columnar seedlings (Sosa Pivatto et al. 2014).

Statistical analyses

We calculated the Pearson correlation coefficient to analyze the relationship between altitude, seed mass, and the different measures of seedling size (height, width and shape index), using the stats packages in R software vs. 2.15.1 (R Development Core Team 2012). In all cases, data met the assumptions of normality and homoscedasticity.

Results

Seed mass was not related to altitude or to any of the other variables (Fig. 2). Seedling height increased significantly with altitude (Fig. 2). Shape index increased significantly with altitude (i.e., seedlings became more columnar with increasing altitude). Seed mass was not related to any variable. Shape index was positively correlated to seedling height and negatively correlated to seedling width (correlation coefficient of 0.97and 0.92, $p<0.05$, respectively.). Changes in seedling shape along the gradient are clearly observed in photographs in Figure 3.

Discussion

Contrary to our expectations, we did not find a relationship between seed size and altitude. Although studies performed at the interspecific level reported a clear pattern between latitude, or altitude, and seed size (Baker 1972; Murray et al. 2003; Moles et al. 2007), studies that had analyzed these patterns at the intraspecific level found contrasting results. For example, in the Swiss Alps Pluess et al. (2005) found different seed size increases with altitude in related species but not among populations of a single species. However, in

Himalayan species of the genus *Pedicularis*, Guo et al. (2010) found a decrease in seed size with altitude, both at intra- and interspecific levels; the authors attributed those differences not to altitude *per se*, but to other variables, such as plant size and seed number per fruit. Although in *G. monvillei*, altitude is not a determinant of seed size, at least directly, we cannot discount the possibility that altitude in combination with other factors (e.g. humidity, soil characteristics or even biotic interactions) has an influence on this variable.

In contrast with Sosa Pivatto et al. (2014), we did not find a relationship between seed size and seedling traits. These discrepancies may be due to the lower variation in seed mass at the intraspecific level than at the interspecific level. Although seed size did not vary with altitude, seedling height and shape index showed a very clear altitude-related pattern. Seedling height increased with increasing altitude. Sosa Pivatto et al. (2014) stated that a more columnar seedling would be better competitors for light, but less tolerant to higher temperatures or drought. Our results show that this tradeoff could also operate in seedlings of similar seed sizes in response to marked differences in climate. In the present study, at higher altitudes, in a much cooler and wetter environment, seedlings were found to be columnar, which could be an advantage for light competition (Leishman et al. 2000). On the other hand, at the other extreme of the gradient, a globose morphology would make seedlings more resistant to a hotter and drier environment. In other words, factors associated with altitude (temperature and humidity) would be exerting a strong selective pressure on seedling traits, altering seedling morphology. Interestingly, low temperatures at the higher sites, where frosts occur year round, did not be constraining seedling shape. Physiological, rather than morphological mechanisms are likely operating in protecting seedlings from low temperatures (Nobel 1982). For example, water loss before winter plays an important role in preventing frost damage in *Opuntia* species (Shikawa and Gusta 1996).

Climate change is affecting organisms worldwide, and understanding species-environment relationships would help to predict species responses (Aragón-Gastélum et al. 2014). It could be accepted that species with wide distributions along climatic gradients, such as *G. monvillei*, would be less affected by climate change than narrowly distributed species (Ureta et al. 2012). However, our results suggest that *G. monvillei* populations could be negatively affected by changes in temperature if seedling morphology of the different altitudinal classes remained unchanged. In addition, dispersion in this species is mediated by ants, which are short-distance dispersers (Bregman 1988; Lengyel et al. 2010), and therefore movement of seeds among different populations is not a common occurrence. Cacti diversity across the Americas is concentrated in mountain environments (Mourelle and Ezcurra 1996; Ortega-Baes and Godínez-Alvarez 2006); therefore, understanding how species are distributed and adapted to these environments is very important to predict their responses to environmental changes.

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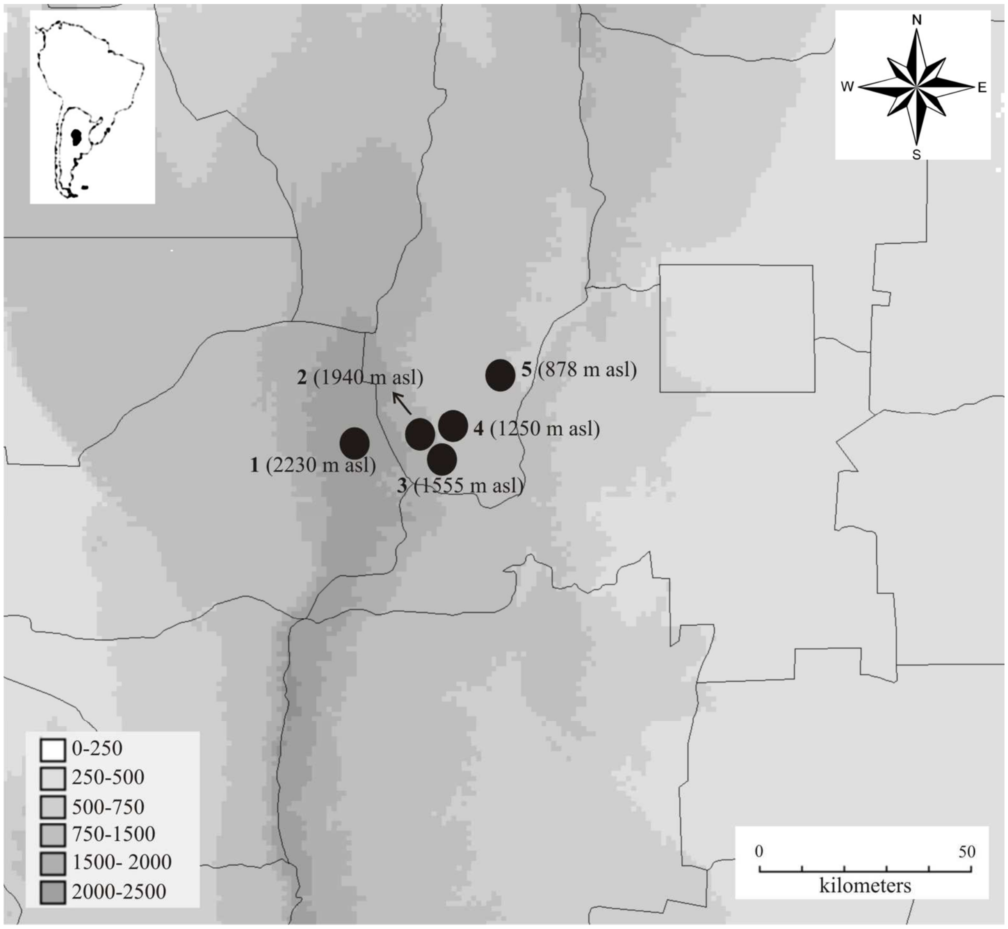
Figure Legends

Fig. 1. Location of the five collection sites of seeds of *Gymnocalycium monvillei* along an altitudinal gradient in e Córdoba Mountains (central Argentina).

Fig. 2. Relationships between altitude and seed mass (a), seedling height (b), seedling width (c), and shape index (d), and between seed mass and seedling height (e), seedling width (f), and shape index (g) in *Gymnocalycium monvillei*. *, $p \leq 0.05$; ns, non-significant; Pearson correlations.

Fig. 3. Photographs of typical seedlings of *Gymnocalycium monvillei* for each altitudinal class.



161x148mm (300 x 300 DPI)

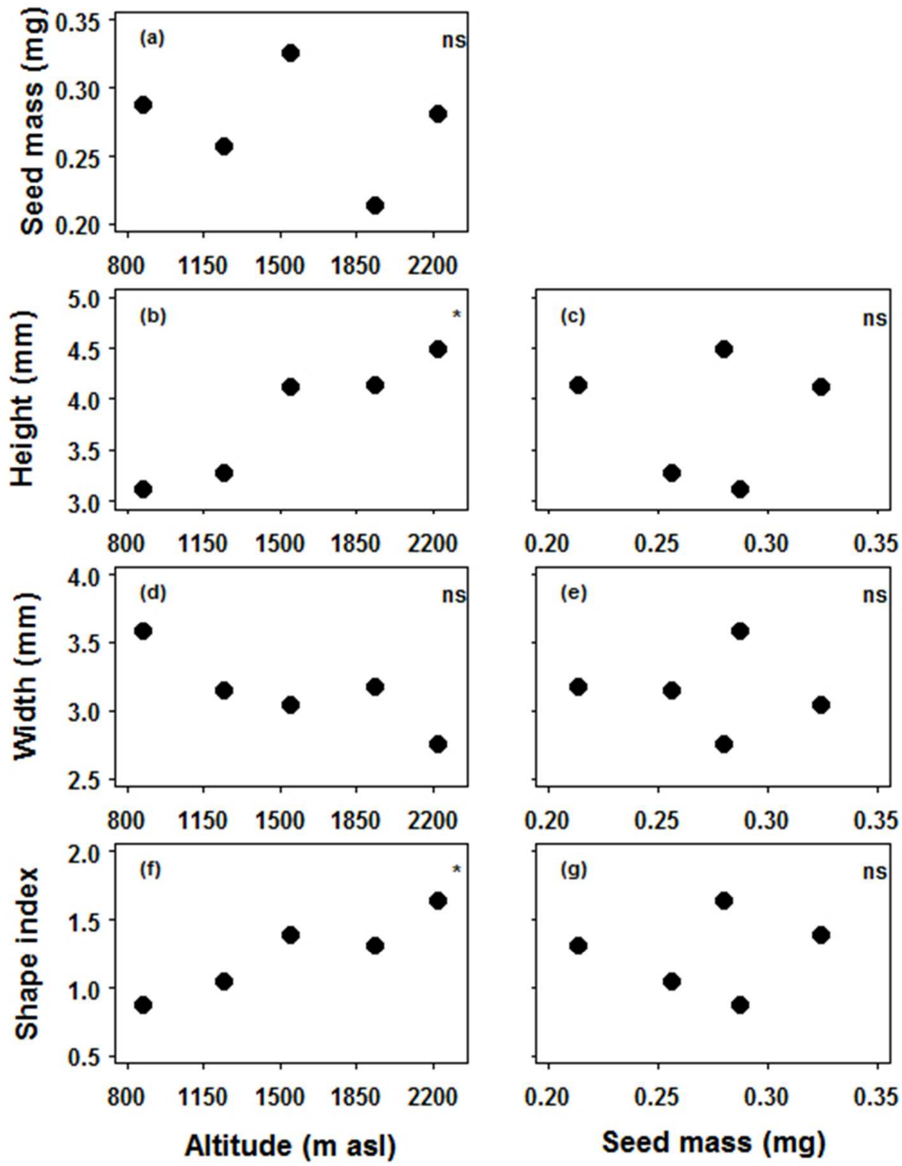


Fig. 2. Relationships between altitude and seed mass (a), seedling height (b), seedling width (c), and shape index (d), and between seed mass and seedling height (e), seedling width (f), and shape index (g) in Gymnocalycium monvillei. *, p≤0.05; ns, non-significant; Pearson correlations.
183x235mm (72 x 72 DPI)

Fig. 3. Photographs of typical seedlings of Gymnocalycium monvillei for each altitudinal class. (THESE PHOTOGRAPHS CAN BE PRESENTED BLACK & WHITE)
152x42mm (300 x 300 DPI)