Flowering phenology, fruit set and seed mass and number of five coexisting *Gymnocalycium* (Cactaceae) species from Córdoba mountain, Argentina

Flowering phenology, fruit set and seed mass and number of five coexisting *Gymnocalycium* (Cactaceae) species from Córdoba Mountain, Argentina[1]

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Key words: cacti, reproductive success, seed mass-number trade-off, sympatric species, synchronicity

Introduction. A central question in ecology is how plant species using similar resources coexist (Leibold *et al.* 2004). Numerous mechanisms have been proposed in order to explain plant coexistence (Chesson 2000, Chase 2005, Wilson 2011). In particular, it has been postulated that species coexisting at reduced spatial scales have to partition the use of resources (Bazzaz 1991, Chesson and Huntly 1997, but see Hubbell 2001), implying a niche differentiation among them (*sensu* Hutchinson 1951). In this sense, flowering phenology has been proposed historically as a key reproductive trait influencing the local coexistence of flowering plants, since flowering at different times may allow the temporal partitioning of resources, such as pollinators or fruit dispersers, among sympatric plants (Elzinga *et al.* 2007). However, flowering phenology may also promote plant coexistence

by influencing key traits related to dispersal ability (*i.e.,* seed number) and/or the regeneration niche of species (*i.e.,* seed mass; Moles and Westoby 2003). Although the importance of considering the link between these two different roles of flowering phenology in promoting plant coexistence has been highlighted in the past (Primack 1987), studies focusing on only one of these roles still prevail over studies combining both factors (Chuine 2010, Forrest and Miller-Rushing 2010).

The temporal sequence of flowering, fruit production, and seed dispersal may be particularly important in seasonal climates because the length of the favorable season limits growth, flowering, seed development, and seed dispersal (Moles and Westoby 2006, Du and Qi 2010). Flowering time (*i.e.,* the time of the season when flowering occurs) influences the time of occurrence of subsequent phenological phases and therefore may affect plant fitness and, ultimately, plant coexistence (Primack 1987, Forrest and Miller-Rushing 2010). Flowering time may have important consequences for the final number of seeds available for dispersal (Primack 1987, Moles and Westoby 2003). For instance, the seed production of species flowering before their pollinators emerge may be pollen limited and/or may be disproportionally decreased by the presence of floral pathogens or seed predators (Elzinga *et al.* 2007). Moreover, unfavorable abiotic conditions, such as frost events at the beginning or end of the growing season, may strongly affect the survival of seedlings of flowering plants (Elzinga *et al.* 2007, Chuine 2010). However, it has been proposed that plants flowering earlier in the season have more time for fruit maturation and thus, other things being equal, these plants may present higher fruit set than plants flowering later (Primack 1987). Moreover, early-flowering species may produce larger seeds, as they have a longer time for seed development (Eriksson and Ehrlén 1991, Moles and Westoby 2003). In contrast, late-flowering species would have a shorter seed development time and therefore produce smaller seeds ensuring enough time for the subsequent phenological stages (Eriksson and Ehrlén 1991, Moles and Westoby 2003). However, other studies failed to show any clear tendency in the association between flowering time and seed mass (*i.e.,* Bolmgren and Cowan 2008, Du and Qi 2010).

Independent of the association of seed traits with flowering time, the evolutionary trade-off between seed mass and seed number (Coomes and Grubb 2003, Turnbull *et al.* 2004, Ben-Hur *et al.* 2012) may explain the coexistence of plants. This trade-off implies that species having lighter seeds tend to produce more seeds than species with heavier seeds. Both phenologies (numerous and light vs. few and heavy seeds) may be advantageous for success in colonization and persistence. In general, seedlings from species with heavier seeds show higher survival in stressful environmental conditions (Bowers and Pierson 2001, Westoby *et al.* 2002). However, these species tend to produce fewer seeds and therefore their dispersal may be numerically and spatially restricted (Turnbull *et al.* 1999). In contrast, light-seeded species may produce potentially more seeds and thus show higher dispersal abilities. However, their seedlings are more susceptible to unfavorable environmental conditions during establishment (Turnbull *et al.* 1999, Bowers and Pierson 2001, Turnbull *et al.* 2004, Leck, Parker, and Simpson 2008, Ben-Hur *et al.* 2012, Sosa Pivatto *et al.* 2014).

In cacti species with low growth rates and restricted geographical distribution, the reproductive and regenerative traits may play an important role in determining population dynamics and thus in allowing species coexistence (Petit 2001, McIntosh 2002, Mandujano *et al.* 2010, Sosa Pivatto *et al.* 2014). The genus *Gymnocalycium* (Cactaceae) comprises about 50 species distributed in southern South America (from Bolivia, Paraguay, southern Brazil, and Uruguay to central Argentina; Charles 2009). All species present a globose body, and may be solitary or colonial. The mountains in Córdoba province (central Argentina) are an important diversity center for *Gymnocalycium* with around 17 species, most of them endemic (Zuloaga and Morrone 1996, Gurvich *et al.* 2014). Five of these endemic species coexist locally in the Córdoba Mountains (Gurvich, Demaio, and Giorgis 2006) and thus represent a particularly interesting biological system in which to study plant coexistence. Moreover, a comparison of plant traits that may allow plant coexistence in species from the same genus may minimize the interspecific differences in those traits that are due to phylogenetic constraints and/or evolutionary history, even if the species are not
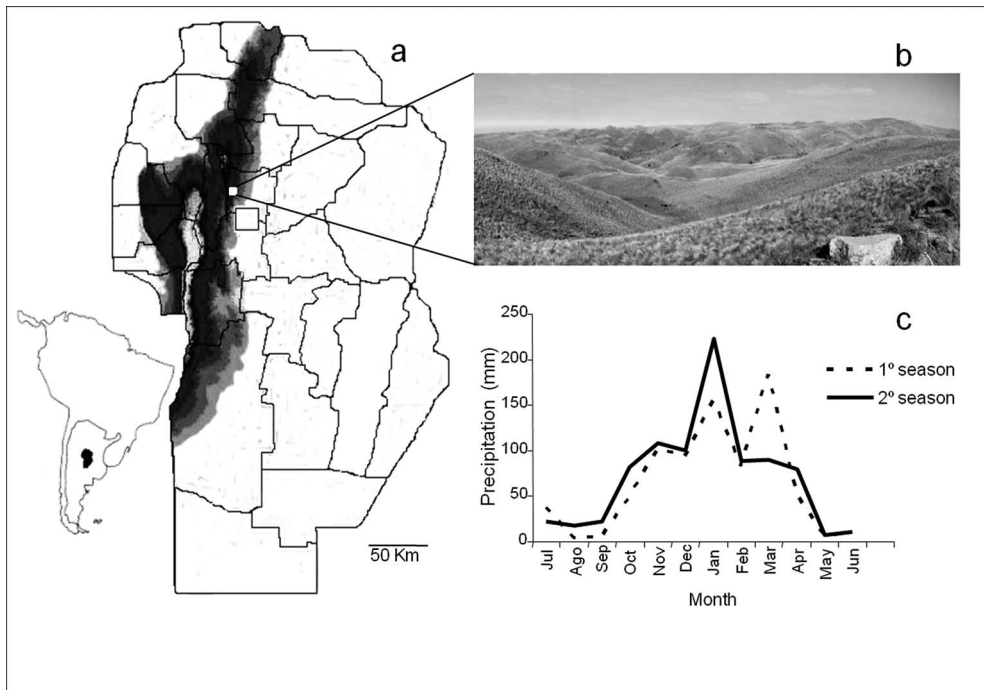
Fig. 1. (a) Location of the study area in the mountains in Córdoba province (central Argentina). (b) Precipitation (mm) during the study period (the dotted line indicates the first season and the continuous line, the second season).

closely related, as compared to studying the entire community.

In order to improve our understanding of how these five *Gymnocalycium* species coexist in the mountains in Córdoba, we compared the flowering phenology, seed production, and seed mass of the different species. Specifically, we analyzed how flowering phenology was related to seed mass and number, and the relationship of the seed traits in these sympatric species. In addition, as phenological patterns may change between years (Rathcke and Lacey 1985, Petit 2001, Munguía-Rosas and Sosa 2010), we measured all these plant traits in two consecutive flowering seasons.

Materials and Methods. STUDY SITE AND SPECIES. The study was carried out in an area of ca. 40 ha, located on the eastern slope of the Sierras Chicas mountain range (Córdoba province, Argentina, 31°07′S, 64°24′W), at 1,200 m a.s.l., in a private ranch called La Sureña (Fig. 1a, b). Climate is temperate and subhumid, with a mean annual temperature of 13.9°C (Hijmans *et al.* 2005) and a mean annual precipitation of 950 mm with most rainfall concentrated in the warmest months, from October to April, showing high interannual variability (range 600–1,500 mm at 700 m a.s.l. in the period 1960 to 2006; J.J. Filardo, Affiliation, unpublished data). Snowfalls have been occasionally recorded, representing an insignificant amount of the total precipitation in the study area. Rainfall was higher during the second study season than during the first (Fig. 1c). The vegetation of the study area is a mosaic of grasslands, shrublands, woodlands and rocky outcrops (Gurvich *et al.* 2006) subjected to cattle grazing and exposed to recurrent fires (Gavier and Bucher 2004).

Five *Gymnocalycium* species of three different subgenera are present in the study area: *Gymnocalycium bruchii* (Speg.) Hoss. and *Gymnocalycium capillense* (Schick.) Backerb. of the subgenus *Gymnocalycium*; *Gymnocalycium quehlianum* (F.Haage × Quehl) Vaupel ex Hosseus of the subgenus *Trichomosemineum*; and *Gymnocalycium monvillei* (Lem.) Britton et Rosse and *Gymnocalycium mostii* (Gürke) Britton et Rosse of the subgenus *Scabrosemineum* (Demaio *et al.* 2011). The abundance of the five species in this area is: *G.*

bruchii (1.76 indiv./m²); *G. capillaense* (0.52 indiv./m⁻²); *G. quehlianum* (0.17 indiv./m⁻²); *G. monvillei* (0.36 indiv./m⁻²), and *G. mostii* (0.15 indiv./m⁻²; Gurvich, Giorgis, *et al.* 2008; D.E.G., unpublished data).

All species are self-incompatible (Ganders 1976, Charles 2009) and offer both pollen and nectar as rewards to pollinators (J.A. and A. Cosacov, Affiliation, unpublished data). Pollinators are mainly species of bees of the Halictidae family (J.A. and A. Cosacov, Affiliation, unpublished data). Flowers from these species have a lifespan of between two to three days (M.A.G., unpublished data). Fruits of all species are fleshy, oval, or spherical, and reach maturity in approximately five weeks (M.A.G., unpublished data). Seeds bear an elaiosome, which attracts different ant species that act as seed dispersers. The most frequently observed species in this area are *Prenolepis* sp., *Camponotus rufipes*, and *Crematogaster quadriformis* (M.A.G., unpublished data).

The study was carried out during two flowering seasons: from July 2004 to March 2005, and from July 2005 to March 2006 in the first and the second study seasons, respectively. In winter 2004 (July–August), we selected and tagged reproductive individuals of four out of the five species: 44 individuals of *G. bruchii*, 40 of *G. quelhianum*, 36 of *G. monvillei*, and 33 of *G. capillense*. In July 2005, additional individuals of all four species were marked, totaling 58, 56, 42, and 52, respectively. Additionally, in 2005 we selected and tagged 51 individuals of *G. mostii*, the species that was not measured during the first study season. No individuals died during the first season, whereas the death of individuals of four species was recorded during the second season (six individuals of *G. monvillei*, one of *G. capillense*, two of *G. bruchii*, and three of *G. mostii*).

FLOWERING PHENOLOGIES. We visited each tagged individual every 5 to 15 days, from the beginning to the end of the flowering season, in the two study seasons. Visits were more frequent during flowering peaks (November to February).

During each visit, we recorded the following information for each individual: number of newly initiated buds (*i.e.,* those not detected in previous visits), aborted buds, open flowers, aborted flowers, mature fruits, and aborted fruits. At the end of each season, total

numbers for these variables were obtained by adding up the values obtained for each individual throughout the visits. Aborted buds and flowers were easy to distinguish due to their different sizes and by the presence of wilted petals in the case of flowers.

To characterize the flowering phenology of each individual for each study season, we estimated the following phenological variables: onset (*i.e.,* the date at which the first flower was opened) and flowering peak (*i.e.,* the date at which the highest number of open flowers was recorded), both of which describe the timing of the flowering phenology of each individual, and flowering duration (*i.e.,* the number of days between the dates at which the first and the last flower were opened).

We obtained the temporal overlap (synchronicity) between the bloom of each individual of each species and those of the other species studied, by calculating a modified version of the index proposed by Elzinga *et al.* (2007) as follows:

$$S = \sum_{t=1}^{k} \left(\frac{x_t}{n} \right) p_t, \qquad (1)$$

where S is the temporal overlap index for each individual, x_t is the number of flowers of the focal individual on visit t, n is the total number of flowers of that individual throughout the season, k is the total number of visits in which the individual showed open flowers, and p_t is the proportion of individuals of the other species pooled that had open flowers at that visit. Then, highest S values are associated with higher flowering overlap between the focal individuals and their sympatric congeners (*i.e.,* individuals of the other species but not of the same species).

FRUIT SET, SEED MASS, AND NUMBER. We calculated individual fruit set as the ratio between the total number of mature fruits and the total number of open flowers produced by each individual. In order to characterize seed mass and number during the first flowering season (2004–05), we collected all undamaged mature fruits produced per species, whereas during the second season (2005–06), we only collected half of them at random. We collected 32 and 4 fruits of *G. monvillei*, 23 and 15 of *G. bruchii*, 19 and 12 of *G. quehlianum* in the first and second seasons, respectively, and 4 fruits of *G. mostii* in the second season. We

collected only four fruits of *G. capillense* in the first season, whereas in the second season, this species did not produce any fruits. The collected fruits were air-dried to facilitate seed cleaning. We counted all seeds per fruit. Total seed number produced by an individual plant in one year was obtained as the product between the number of seeds per fruit and the number of mature fruits per plant. Seed mass was calculated by dividing the total seed mass of individual fruits by the number of seeds.

DATA ANALYSES. We performed size frequency distributions to avoid the effect of individual cactus size on the reproductive traits evaluated (*e.g.,* McIntosh 2002, Mandujano *et al.* 2010). We defined five size classes for each species, and selected all individuals belonging to the three intermediate categories to ensure an adequate size distribution. Based on this selection, we analyzed data from 25 and 31 individuals of *G. monvillei*, 35 and 48 of *G. quelianum*, 24 and 42 of *G. capillense*, 44 and 58 of *G. bruchii* (first and second season, respectively) and 47 of *G. mostii* during the second season.

We compared onset, duration, flowering peak, synchronicity, the total number of buds, aborted buds, open flowers, aborted flowers, mature fruits, and aborted fruits per individual among species using Kruskal-Wallis and Mann-Whitney tests. These tests were chosen because the distributions of the residuals differ from the normal distribution when using parametric statistics. Differences in the number of seeds per fruit and seed mass were analyzed through one-way ANOVA and Tukey's *a posteriori* tests. To compare all these phenological and reproductive variables among the five species, we used the data obtained in the second study season, except for seed number and seed mass of *G. capillense* for which we used data from the first season. In order to describe differences in flowering patterns between years, we performed within-species comparisons between seasons for all species except *G. mostii*, for which we had no data for the first season. For the within-species comparisons between seasons, we performed Mann-Whitney tests for the number of buds, aborted buds, open flowers, aborted flowers, mature fruits, and aborted fruits, and ANOVA for the number of seeds per fruit and seed mass. We could not perform the latter comparison for *G. capillense*.

To analyze the main patterns of variation between species at flowering time, fruit set, and seed mass–number trade-off across the species in reproductive traits, we performed a multivariate analysis (PCA). In this analysis, we included onset (related to the start of the flowering period), reproductive success, and the number of seeds per fruit and seed mass.

Results. FLOWERING PHENOLOGIES. *Gymnocalycium bruchii* showed the earliest onset and the earliest flowering peak, differing significantly from the onset and peak of the other four species. However, in these four species, the onset and flowering peak were similar (Fig. 2, Table 1). Flowering duration was significantly shorter for *G. capillense* and *G. bruchii*, intermediate for *G. monvillei*, and longer for *G. mostii* and *G. quehlianum* (Table 1). Flowering synchronicity was lower for *G. capillaense* and *G. bruchii*, intermediate for *G. mostii* and *G. quehlianum*, and higher for *G. monvillei* (Table 1).

FLOWERING CHARACTERIZATION. The total number of initiated buds per individual was significantly higher for *G. quehlianum* and *G. mostii,* intermediate for *G. bruchii* and *G. monvillei.* and lower for *G. capillense* (Table 2). The number of aborted buds was significantly higher for *G. quehlianum*; intermediate for *G. mostii, G. monvillei,* and *G. capillense*; and lower for *G. bruchii*. The number of open flowers per individual was significantly higher for *G. quehlianum*, intermediate for *G. monvillei, G. mostii,* and *G. bruchii*; and lower for *G. capillense*. The number of aborted flowers was significantly higher for *G. mostii* and lower for *G. capillense*, whereas *G. bruchii, G. quehlianum,* and *G. monvillei* presented intermediate values. Mature fruits were significantly higher for *G. quehlianum*; intermediate for *G. monvillei, G. mostii,* and *G. bruchii*; and lower for *G. capillense*; however, there was no significant difference between *G. quehlianum* and *G. monvillei*. No significant differences were found in the number of aborted fruits among species (Table 2).

FRUIT SET, SEED MASS, AND NUMBER. The percentage of individuals producing flowers varied among species, with 85.7% for *G. quehlianum*, 72.4% for *G. bruchii*, 57.4% for *G. mostii*, 38.1% for *G. monvillei*, and 25.5% for *G. capillense*. Fruit set was significantly higher for *G. quehlianum*; intermediate
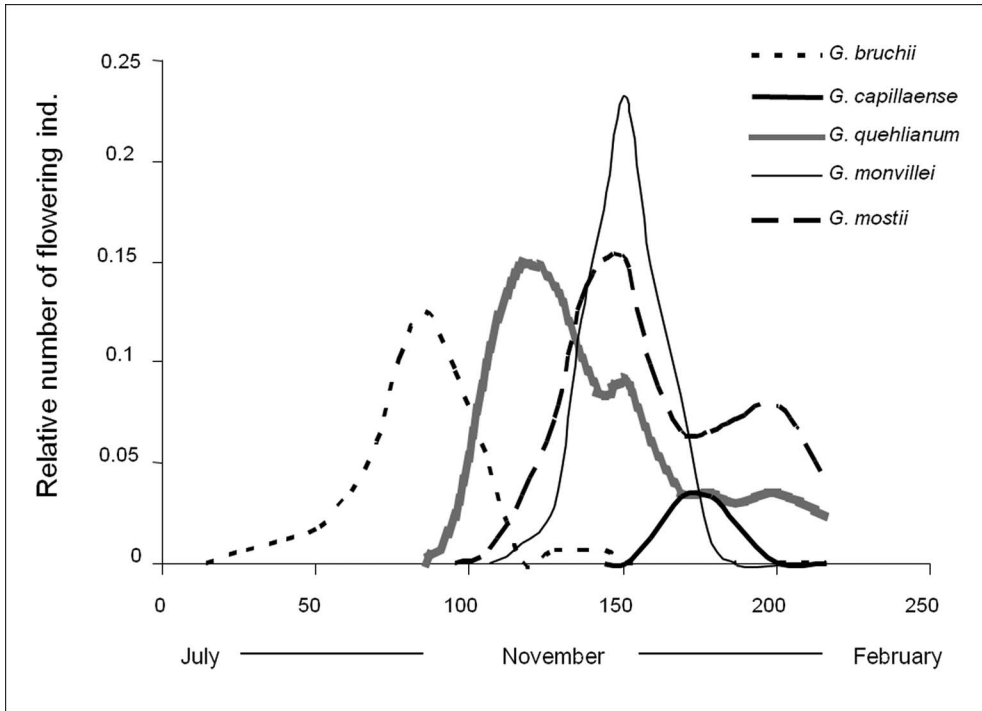
FIG. 2. Flowering phenologies for each species in the second season; the relative number of individuals in flower per species is plotted throughout the season. Patterns were constructed with moving averages. Dates are given in Julian date format.

for *G. bruchii*; and lower for *G. monvillei*, *G. mostii*, and *G. capillense*, whereas no significant differences were found between *G. bruchii* and *G. mostii* (Table 2).

The number of seeds per fruit was only significantly higher for *G. monvillei,* whereas the other species presented no differences (Table 2). Within each species, the number of seeds per fruit was highly variable among individuals of species (Table 2); for instance, it ranged from 8 to 208 seeds per fruit in *G. bruchii,* and from 409 to 4,014 in *G. monvillei*. The average number of seeds produced by an individual in one season was significantly higher for *G. monvillei*; intermediate for *G. quehlianum*; and lower for

G. mostii, *G. capillense*, and *G. bruchii* (Table 2). *Gymnocalycium bruchii* and *G. capillense* had significantly heavier seeds than the other three species (Table 2). The seed mass was less variable within species than the number of seeds per fruit (Table 2).

ONSET, FRUIT SET, AND SEED SIZE– NUMBER TRADE-OFF. The multivariate analysis revealed the relationships among onset, fruit set, and the seed size–number trade-off across the five species (Fig. 3). The first two PCA axes accounted for 96.9% of the variance (68.8% and 28.1% for axes 1 and 2, respectively). The species that produced a low number of heavy seeds per fruit and lower

Table 1. Flowering parameters for each species in the second season: onset, duration, flowering peaks, and synchronicity. Dates are given in Julian date format. Different letters indicate statistically significant differences among species (Kruskal-Wallis test).

Species	Onset	Duration	Flowering peaks	Synchronicity
Gymnocalycium monvillei	143.9 bc	11.4 ab	149.1 b	0.15 c
Gymnocalycium mostii	154.4 c	32.1 b	177.9 b	0.13 bc
Gymnocalycium quehlianum	133.3 b	36.3 b	148.6 b	0.12 bc
Gymnocalycium capillense	175.6 c	3.0 a	175.6 b	0.05 ab
Gymnocalycium bruchii	82.44 a	9.1 a	86.7 a	0.01 a

Table 2. Mean number of initiated and aborted buds, open and aborted flowers, mature and aborted fruits per individual, number of seeds per fruit, seed mass and fruit set (data for the second season). The maximum value recorded is indicated in parentheses. Different letters indicate statistically significant differences among species (Kruskal-Wallis test), and ANOVA with Tukey's a posteriori test for number of seeds (per fruit and individual) and seed mass (mg).

Species	Initiated buds	Aborted buds	Open flowers	Aborted flowers	Mature fruits	Aborted fruits	Fruit set	Number of seeds per fruit	Number of seeds per individual	Seed mass (mg)
Gymnocalycium monvillei	2.62 (9) ab	1.31 (4) ab	1.13 (7) b	0.31 (3) ab	1 (7) ab	0.1 (1) a	0.28 a	1,439 (4,014) a	1,252 (3,492) a	0.24 (0.43) b
Gymnocalycium mostii	3.65 (15) c	1.91 (9) bc	1.74 (11) b	0.5 (5) b	1.24 (6) b	0.26 (3) a	0.39 ab	659 (935) b	870 (1,234) b	0.22 (0.27) b
Gymnocalycium quehlianum	3.82 (12) c	1.64 (6) c	2.18 (6) c	0.18 (2) ab	2 (6) c	0.02 (1) a	0.77 c	359 (1,018) b	703 (1,995) ab	0.20 (0.29) b
Gymnocalycium capillense	0.98 (3) a	0.73 (3) ab	0.25 (1) a	0.06 (1) a	0.2 (1) a	0.04 (1) a	0.2 a	295 (410) b	93 (193) b	0.71 (0.90) a
Gymnocalycium bruchii	1.62 (7) b	0.52 (3) a	1.1 (4) b	0.26 (2) ab	0.84 (4) b	0.09 (1) a	0.53 b	100 (208) b	62 (86) b	0.67 (1.20) a

fruit set (*G. capillense*) were at the positive extreme of axis 1. The other species, *G. quehlianum*, *G. mostii*, and *G. monvillei,* with opposite characteristics, were located at the negative extreme. The species with an earlier flowering peak (*G. quehlianum* and *G. bruchii*) were located at the negative extreme of axis 2, whereas the two species with opposite characteristics (*G. mostii* and *G. capillense*) were located at the positive extreme (Fig. 3).

INTER-ANNUAL VARIATION WITHIN SPECIES. *Gymnocalycium monvillei* had significantly more aborted fruits per individual in the first season (0.48, range 0–4) than in the second (0.03, range 0–1, $P = 0.008$) and *G. capillense* had significantly more aborted flowers per individual in the first season (0.25, range 0–2) than in the second (0.05, range 0–2, $P = 0.04$). Differences in the number of initiated buds, flowers and fruit production between the two study seasons were not statistically significant for any of the four species compared (data not shown). Flowering phenologies were consistent in both seasons for the four species, with all species showing a delay in the onset of flowering in the second one (see supplemental material).

Discussion. Flowering phenology may be a key biological trait for understanding plant coexistence, since it may influence not only reproductive success but also its regeneration success (by affecting seed mass) and dispersal (by influencing seed number; Primack 1987, Moles and Westoby 2003, Chuine 2010). In this study, we compared flowering phenology, reproductive traits, and regenerative traits in five coexisting species. We found that the earliest flowering species tend to have higher reproductive success (higher fruit set) than species flowering later, but we did not find a clear relationship between the flowering time and seed number or seed mass. However, species producing heavier seeds showed lower seed production than species producing lighter seeds. Thus, a trade-off between colonization versus competitive ability (Levin and Peine 1974, Tilman 1994, Barot 2004) may be important in explaining the coexistence of these species of cacti.

The time of the season when cacti species started to flower affected the success of flowers developing into fruits (fruit set). In agreement with the idea that early flowering
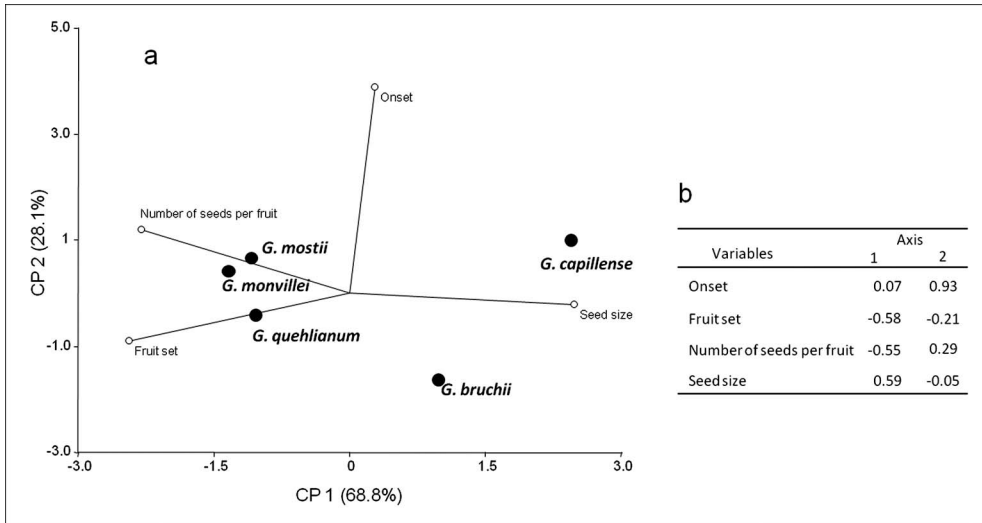
Fig. 3. Principal component analysis of the five *Gymnocalycium* species on the basis of reproductive variables and seed characteristics. (a) Biplot for the first and second axes. (b) Auto-vectors for the first two axes.

species may have an adequate amount of time for fruit to mature (Eriksson and Ehrlén 1991, Moles and Westoby 2003), the earliest flowering species tended to have the highest fruit set (*G. bruchii* and *G. quehlianum*). In contrast, the later flowering species, *G. capillense*, showed the lowest values of fruit set, which may be explained by a reduction in the time for fruit maturation due to the lower temperature that characterized the end of the growing season (Fig. 2, Table 2). However, the higher success of early flowering species may also be associated to other factors affecting reproductive success, such as pollinator availability, and/or the presence of flower pathogens or fruit predators (Levin 2006, Elzinga *et al.* 2007, and their references). Species flowering asynchronously may minimize the transference of incompatible pollen, avoiding stigma clogging and therefore maximizing reproductive success (Primack 1980, Fenner 1998, Elzinga *et al.* 2007). However, pollinator activity is clearly conditioned by climatic conditions, being lower at both extremes of the growing season (Elzinga *et al.* 2007). Moreover, many studies found that off-peak flowering in the community may be associated with a reduction in seed predation, probably due to lower herbivore densities and/or a reduction in pollinator-transmitted diseases, due to the lower pollinator activities at those times (Levin 2006). As we found different flowering times among species, we suggest that the use of pollinator resources is partitioned between the species with different consequences in their reproductive success. Some of these five congeneric species living in sympatry may flower in isolation ensuring an adequate pollen transfer (*G. bruchii* and *G. capillense*) but may be under hard climatic conditions or low pollinator activity, while other species may flower at the same time during favorable climatic conditions and higher pollinator activity (*G. monvillei* and *G. mostii*), but with the risk of inadequate pollen transfer or higher pressure from flower pathogens or seed predators.

In this context, the flowering phenology of *G. quehlianum* may explain its higher reproductive success and its higher number of fruits, as this species flowered early with an asynchronic flowering peak and continued to flower during the middle of the season. Interestingly, even though *G. quehlianum* showed the highest fruit set, the highest total number of fruits per individual and the highest germination percentage of the species studied (Gurvich, Funes *et al.* 2008), it is the least abundant in the study region (0.17 indiv./m²; Gurvich, Giorgis *et al.* 2008). Therefore, we suggest that other stages of the plant life cycle (*e.g.,* seedling establishment) may be key in determining the local abundance of the species studied and, ultimately, their coexistence.

Larger seeds have been found in early flowering plants of perennial herbs in ecosystems with a marked growing season (*e.g.,* Vile *et al.* 2006, Bolmgren and Cowan 2008, Du and Qi 2010). These findings support the idea that larger seeds require longer development time (Eriksson and Ehrlén 1991, Moles and Westoby 2003), which may lead to earlier flowering times in seasonal climates. Nevertheless, our results showed inconsistent patterns as the species with larger seeds, *G. bruchii* and *G. capillense,* flowered at the beginning and at the end of the growing season, respectively. Since these species are closely related (belonging to the same subgenera; Demaio *et al.* 2011) these results may indicate that seed mass in the species studied is highly conditioned by other factors associated with the evolutionary history of these species and not by ecological factors related to seasonality. The close relationship between seed characteristics and taxonomy in the genus *Gymnocalycium* was suggested in traditional botanical studies many years ago (*e.g.,* Kreuzinger 1935, Buxbaum 1968) and is highly supported by molecular studies more recently (Meregalli, Ercole, and Rodda 2010, Demaio *et al.* 2011).

We found a strong relationship between seed mass and number in these species (Fig. 3). Given that a species has a certain amount of resources for reproduction, the species may produce either a low number of large seeds leading to highly competitive seedlings, or a higher number of small seeds leading to more vulnerable seedlings (Primack 1987, Turnbull *et al.* 1999). Species producing a few large seeds may be numerically restricted for dispersal to suitable regeneration microsites, whereas species with numerous smaller seeds may be more successful in reaching those microsites (Turnbull *et al.* 1999, Fenner and Thompson 2005). Turnbull *et al.* (1999) suggest that although seedlings from small-seeded species are less successful in direct competition for regeneration microsites, the fecundity advantage of these species ensures that they will be present in a higher percentage of regeneration sites and will become established in sites where larger seeds are absent. Thus, coexistence may depend critically on allocation trade-offs that prevent species from being both good competitors and good colonizers (Turnbull *et al.* 1999). This may be the case of the species studied as they are coexisting in a small area, but they are segregated at the microscale (in patches of 20 to 100 m; Gurvich, Giorgis, *et al.* 2008). For example, *G. bruchii* grows in sites with higher vegetation cover and deep soil, whereas *G. monvillei* is associated with outcrops, and *G. mostii* occurs in sites with exposed rock and lower vegetation cover (Gurvich, Giorgis *et al.* 2008). In this context, seed arrival and seedling establishment may be key in determining their local abundance and ultimately species coexistence (Barot 2004).

Although we only have data for two years for interannual comparison, our results suggest that the flowering phenology of the different species were almost similar between seasons, differing only in the flowering onset. The similar between-year flowering patterns suggest that flowering phenology might be conditioned by intrinsic factors in the *Gymnocalycium* species, as has been found in numerous species from other families (Ollerton and Lack 1992, Fenner 1998, Davis *et al.* 2010). Between-year differences in the flowering time may be related to the earlier start of the precipitation period during the second season, suggesting that climatic conditions are conditioning the timing of flowering. These changes in flowering time related to differences in seasonality between years reinforce the critical role of flowering time in seasonal climates (Primack 1987). Our study area is characterized by a large interannual variation in precipitation (between 600 and 1,500 mm; J.J. Filardo, unpublished data). Therefore, major changes in the flowering time, survival, and consequently in population and community dynamics, might be expected with changes in the precipitation regime, suggesting a largely dynamic system over the years. These consequences may be of critical relevance in the current context of climate change, particularly in species coexistence at a reduced spatial scale, as it may drive the system to important differences in plant abundance (Chuine 2010, Davis *et al.* 2010).

Conclusion. We found a temporal segregation among the five species suggesting that flowering time is an important trait promoting a temporal separation of resource use that may promote plant coexistence (Barot 2004, Wilson 2010). However, the trade-off between seed mass and number seems to be the most important relationship explaining the coexistence of these species. This trade-off may

result in different dispersal *versus* establishment abilities among species (Turnbull *et al.* 1999, Moles and Westoby 2003, Barot 2004, Wilson 2010). However, further studies concerning seed dispersal ability, seedling survival and the effect of clonality on sexual reproduction and demographic dynamics (Mandujano *et al.* 2010) will certainly improve our understanding of the coexistence of cactus species in our study area.

Supplemental Material. Variation in flowering phenologies: (a) *Gymnocalycium quehlianum*, (b) *Gymnocalycium bruchii*, (c) *Gymnocalycium capillense*, and (d) *Gymnocalycium monvillei,* the relative number of individuals in flower per species is plotted throughout the season. Dotted line indicates the first season; continuous line, the second season. Dates are given in Julian date format.

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