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How common is self-incompatibility across species of the herkogamous genus *Ariocarpus*?[1]

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- *Premise of the study:* Self-incompatibility (SI), the most effective mechanism to prevent selfing, may limit the number of compatible mates in populations. The seven species of *Ariocarpus* are endangered and predominantly outcrossers but fruit set may reach 1–20% after selfing. We aimed to determine whether SI is the underlying mechanism influencing mating in *Ariocarpus* species.
- *Methods:* We characterized the presence/absence of SI using pollination treatments (self-pollination, cross-pollination, natural pollination) in one population per species. We assessed SI using epifluorescence and generalized linear models (GLMs) to compare the presence of pollen tubes in the stigma, stylar transmitting tissue, and ovary among self- and cross-pollinated pistils 48 h after pollination. Following the same treatments, production of fruit set was noted and related to pollen tube growth.
- *Key results:* Pollen tubes were found more frequently in the ovaries of natural and cross-pollinated flowers than in ovaries of self-pollinated. Stylar rejection of self-pollen indicated gametophytic SI, although pollen tubes reached the ovaries in six species (4–33% of pistils). Fruit set was lower after hand-pollinations than expected from pollen tube observations.
- *Conclusions:* The low percentages of self-compatibility in all species in pollen tube growth and pollination experiments indicated that no species had complete self-sterility, suggesting the presence of partial SI. Reduced fruit set relative to pollen tube production could result from a threshold of insufficient pollination, early-acting inbreeding depression, or resource limitation. The origin of partial SI in *Ariocarpus* could respond to pressures such as pollen limitation and population size.

  Key words: *Ariocarpus*; Cactaceae; endemic species; hand-pollination treatments; natural populations; partial self-incompatibility; pollen tube growth; self-incompatibility.

For plant sexual systems, numerous floral attributes are thought to promote outcrossing and reduce the negative effects of selfing (i.e., inbreeding depression, diminished fitness of progeny generated by selfing compared to that of outcrossing; Darwin, 1859, 1876; Schemske and Lande, 1985; Charlesworth and Charlesworth, 1987). Among these floral attributes, herkogamy and dichogamy are remarkable in varying so widely in their presence and expression across angiosperm species. Both

strategies separate sexes temporally or spatially but cannot always prevent selfing; in fact, species with one or more of these mechanisms may still experience some selfing (e.g., Navarro, 1997; Dai and Galloway, 2011; for reviews: Lloyd and Webb, 1986; Webb and Lloyd, 1986). Consequently, herkogamy and dichogamy may also have evolved to avoid sexual interference (Lloyd and Webb, 1986). Self-incompatibility (SI) is thus the most effective anti-selfing mechanism because genetic recognition and inhibition of self-pollen are often highly effective (de Nettancourt, 1977). About 60% of the angiosperms (Hiscock and Tabah, 2003) among at least 68 families possess some type of SI system (Ferrer and Good-Ávila, 2007; Ferrer and Good, 2012). The most common type of self-incompatibility is gametophytic SI (GSI) where recognition occurs via interactions between the pistil (sporophyte) and the pollen tube (gametophyte); in GSI the site of pollen tube inhibition is typically in the upper one third of the style (zone SI, Fig. 1) (Franklin-Tong and Franklin, 2003). GSI systems are regulated by the gene products of the so-called S-locus, and because rare males have more opportunities to find compatible mates, S-alleles are subject to negative frequency-dependent selection, and populations generally harbor many alleles. The probability that a randomly chosen individual will be compatible with another individual from the same population (mate availability, Byers and Meagher, 1992; Vekemans et al., 1998) is typically lower in small populations. Low mate availability may cause reduced seed set in small populations (e.g., Byers and Meagher, 1992; DeMauro, 1993; Wolf and Harrison, 2001; Fischer et al., 2003; Gaudeul and Till-Bottraud, 2003; Willi et al., 2005; Elam et al., 2007) and increase the risk of local extinction in combination
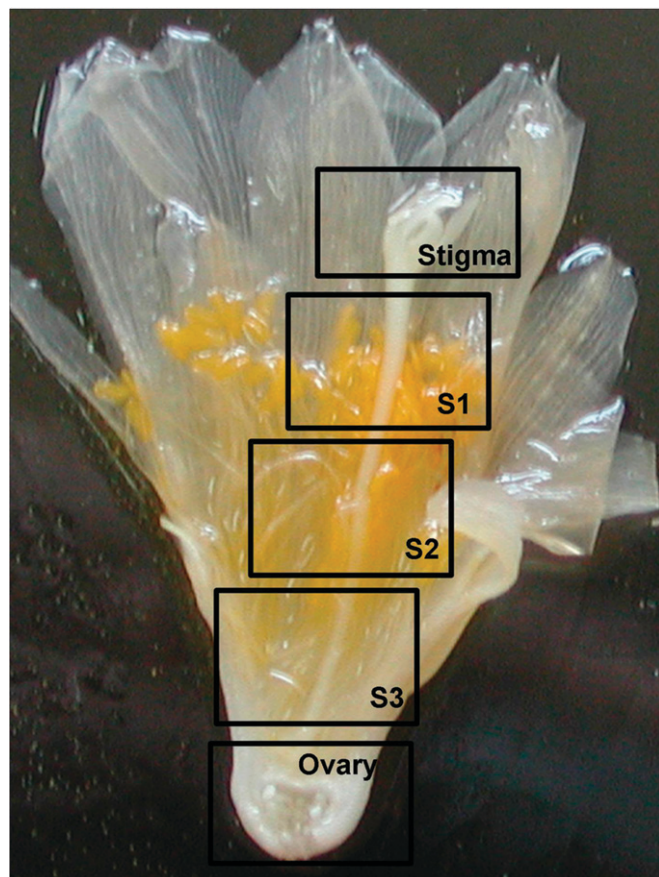
Fig. 1.   Typical flower of *Ariocarpus* (*A. kotschoubeyanus*) in longitudinal section, showing the five zones inspected for pollen tubes.

with demographic and environmental stochasticity, pollinator limitation, and habitat fragmentation (Glémin et al., 2001; Busch and Schoen 2008).

Although research on the reproductive ecology of cacti has increased in recent years, issues such as SI have received little attention (Mandujano et al., 2010). As SI may limit sexual reproduction in natural populations of endangered species, which are common among cacti, knowledge about the dependence of SI on pollinators and compatible mates could help in conservation programs. Floral morphology (perianth size, herkogamy, pollen, and nectar as rewards) and data on fruit and seed set (*Echinomastus erectrocentrus*, Johnson, 1992; *Mammillaria grahammi*, Bowers, 2002; *Ferocactus* spp., McIntosh, 2002; *Opuntia microdasys*, Piña et al., 2007; *Astrophytum asterias*, Strong and Williamson, 2007) suggest the predominance of outcrossing in the Pereskioideae and Cactoideae (Mandujano et al., 2010). Based on these studies, whether outcrossing results from SI (a prezygotic mechanism) or severe inbreeding depression (a postzygotic mechanism) is still unclear (Uyenoyama, 1993).

Studies specifically designed to determine the presence of SI are usually based on the performance of pollen tube growth within the pistil (Lloyd, 1968; Boyle, 1997). As a result of such studies, in members of the Cactaceae, GSI has been identified in selected species of *Schlumbergera*, *Hatiora*, *Echinopsis*, *Hylocereus*, and *Selenicereus*. In *Schlumbergera* and *Hatiora*, self-pollen rejection occurs in the upper part of the style, as in most species with GSI (de Nettancourt, 1997). Conversely, in *Hylocereus* this rejection occurs in the ovary (late-acting SI, Lichtenzveig et al., 2000), but the specific mechanism of recognition is still unclear. In a similar research on *Opuntia tomentosa* (subfamily Opuntioideae) a comparison of pollen tube performance between self- and cross-pollen within pistils revealed full self-compatibility (Galicia-Pérez, 2013).

Transitions from self-incompatibility to self-compatibility that result in high levels of self-fertilization are frequent across angiosperms. These transitions are promoted by lack of pollinators and compatible mates and could reflect changes in life history (Theiss et al., 2010) and floral morphology (Martén-Rodríguez and Fenster, 2008). Understanding these mechanisms within monophyletic groups could shed light into the evolutionary transitions to self-compatibility (e.g., Theiss et al., 2010).

We studied the mechanisms responsible for the low seed production following self-pollination observed in *Ariocarpus*, a monophyletic genus of seven subglobose geophytes endemic to the Chihuahuan Desert and endangered according to Mexican and international laws (Arias et al., 2005). On the basis of fruit and seed set after pollination treatments, *Ariocarpus* species, which are pollinated by solitary bees, are predominantly outcrossers. Nonetheless, selfing can be facilitated (from 1 to 20%, depending on the species (Martínez-Peralta and Mandujano, 2012; C. Martínez-Peralta et al., in press). Our objectives were to determine whether outcrossing is facilitated by self-incompatibility and whether SI is gametophytic or sporophytic in the seven species of the genus by comparing pollen tube growth in different regions of the pistil and fruit set after pollination treatments.

MATERIALS AND METHODS

Species studied—The genus *Ariocarpus* belongs to the tribe Cactaceae within the subfamily Cactoideae. In some populations of *A. retusus*, flowering starts in September, but most species flower during October and early November. In all seven *Ariocarpus* species, flowers remain open 2 or rarely 3 d. Flowers are diurnal, between 2.5 and 4.0 cm in diameter, and emerge at or near the center of the plant. They are white, yellow, or magenta (Fig. 2), depending on the species; color variants within species are also known. Although floral morphology is relatively uniform, flowers of different species produce a significantly different number of male and female gametes and stigmatic lobes (Fig. 2). Flowers of the seven species are incompletely protandrous and exhibit herkogamy, bearing pistils significantly higher than stamens (Fig. 2; Martínez-Peralta et al., in press).

Fieldwork—One locality per species was studied from 2005 to 2010 as follows: *A. agavoides*, in Tula, Tamaulipas (31 October–8 November 2007; 1–4 November 2009); *A. bravoanus*, in Núñez, San Luis Potosí (30 October–2 November 2008); *A. fissuratus* in Cuatro Ciénegas, Coahuila (15–23 October 2005; 25–28 October 2010); *A. kotschoubeyanus* in Congregación San Miguel del Carmen, Tula, Tamps. (31 October–8 November 2007; 1–4 November 2009); *A. retusus* in Miquihuana, Tamps. (4–10 October 2010); *A. scaphirostris* in Rayones, Nuevo León (13–18 October 2010); and *A. trigonus* in Chihue, Tamps. (2–5 November 2007; 23–26 October 2009). We carried out three pollination treatments in each species: selfing, outcrossing, and natural pollination. Manual pollinations, both selfed and outcrossed, were completed between 13:00 and 15:00 hours on both days of anthesis, and flowers remained covered with mesh bags from bud to postanthesis stage. Selfing consisted of manual self-pollinations within flowers with the aid of paintbrushes; flowers in the outcrossing treatment were emasculated before manual cross-pollinations with a mixture of pollen from at least five plants. Naturally pollinated flowers remained uncovered and exposed to pollinators during their complete life span. Pistils were collected and fixed in FAA (10 : 50 : 5 : 35 formalin, 95% ethanol, acetic acid, distilled water) 48 h after the last pollination. Sample sizes varied according to floral availability (from 3 to 66 flowers); the smallest sample sizes
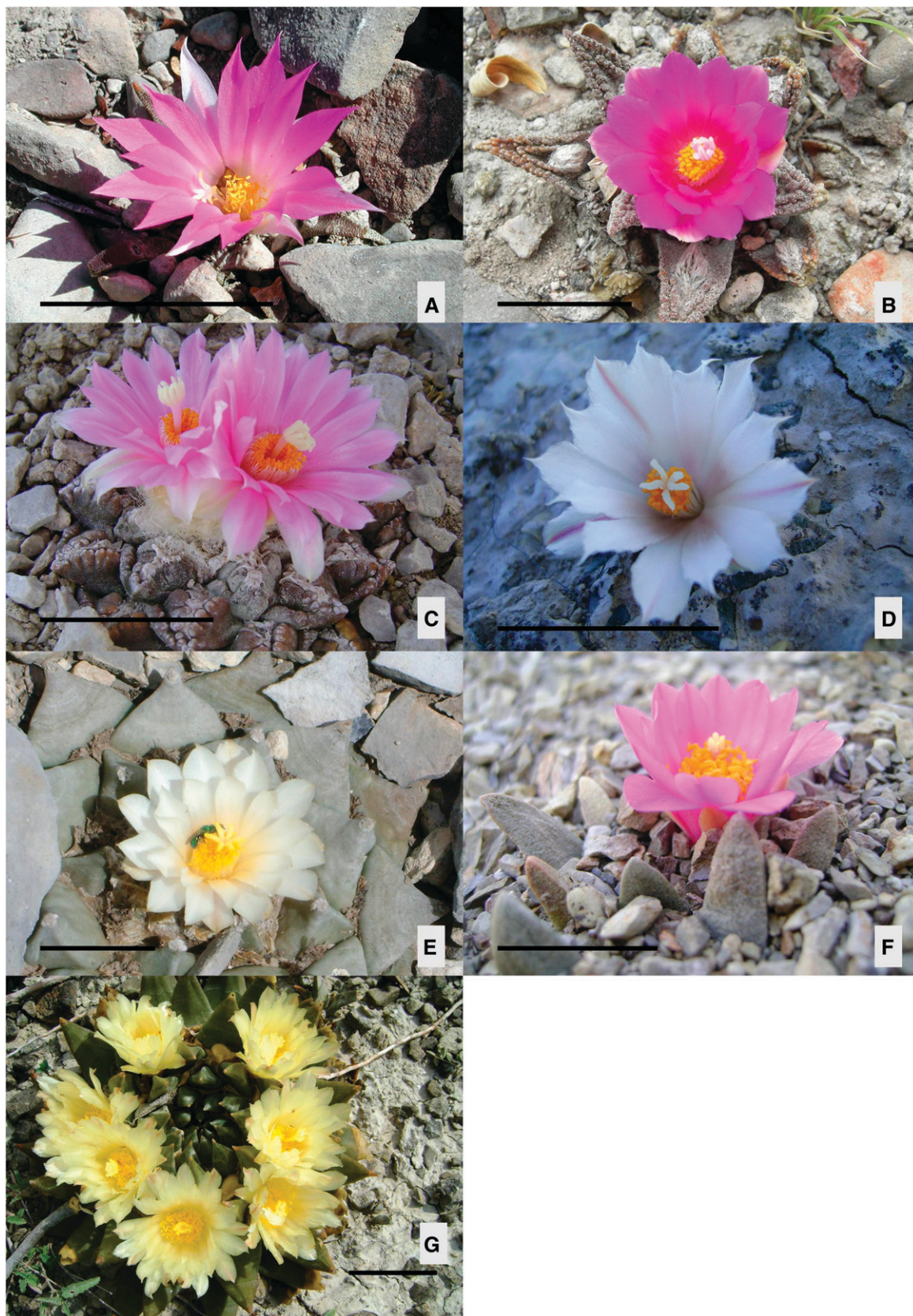
Fig. 2. Flowers of *Ariocarpus* species in their natural habitats. (A) *A. agavoides*, (B) *A. bravoanus,* (C) *A. fissuratus,* (D) *A. kotschoubeyanus*, (E) *A. retusus*, (F) *A. scaphirostris*, (G) *A. trigonus*. Scale bar = 3 cm.

came from *A. bravoanus*, a species under strong pressure of local extinction due to illegal collection (Golubov et al., 2009). The same pollination treatments were conducted to evaluate fruit set; fruits were collected 5–6 mo after pollination. Due to limitations in the sample size of reproductive plants, hand pollinations were carried out during one or two reproductive seasons to achieve larger sample sizes. We collected from 21 to 38 flowers of each species, preserved them in 70% ethanol, and counted the number of ovules per flower with the aid of a stereomicroscope. Differences among species for number of ovules were analyzed using generalized linear models (GLMs) with a Poisson error distribution and log as link function, and differences among species were analyzed with orthogonal contrasts (Crawley, 1993).

Pollen tube observations—Fixed pistils were washed with distilled water to eliminate FAA and softened with 1 N NaOH for 1 h for *A. agavoides* and *A. kotschoubeyanus*, 8 h for *A. scaphirostris* and *A. bravoanus*, and 24 h for *A. trigonus*, *A. retusus*, and *A. fissuratus*. NaOH was eliminated with distilled water, and pistils were stained with 1% (w/v) aniline blue in 0.1 N K_3PO_4. Pistils remained submerged in the staining solution for 24 h in darkness at 4°C. Temporary squash mounts of pistils were observed with epifluorescence microscopy (Olympus BH2-RFCA). In each pistil, we recorded the presence of pollen grains on the stigma and the presence of germinating pollen tubes in five zones from the stigma to the ovary: stigma, stylar zone 1, stylar zone 2, stylar zone 3, and ovary (Fig. 1). We recorded only the presence of pollen tubes since the squash mounts do not allow an accurate count of the tubes. Micrographs were taken with an Olympus C-35AD-4 ASA 100 camera. Pollen tubes were considered as either present or absent within the pistils, and data were compared by means of a GLM with binomial error and logit as a link function in the program JMP v. 7.0 (SAS Institute, Cary, North Carolina, USA). Factors were zone of the pistil that the pollen tubes reached (also including the presence/absence of germinating pollen grains on the stigma) and treatment. To determine the relationship between pollen tube observations and fruit set, we obtained differences between pollen tube production and percentage fruit set (% ovaries with pollen tubes − % fruit set) per treatment of each species. These differences indicated whether fruit set values were those expected from pollen tube observations (difference = 0), lower (positive values), or higher (negative values). Finally, we estimated the ratio of seed to ovule production (seed to ovule ratio) per treatment of each species.

RESULTS

Of 562 flowers analyzed, 96.23% had pollen adhering to the stigma, and 11.47% had pollen adhering but not germinating; thus, 84.76% of flowers had germinating pollen grains (hereafter *pollinated stigmas*; Fig. 3A–D). The mean percentage of pollinated stigmas pooled across species was highest after natural pollination (99.59 ± 0.41, mean ± SE %), intermediate after self-pollination (88.89 ± 3.99 mean ± SE %), and lowest after cross-pollinations (75.91 ± 7.20, mean ± SE %). *Ariocarpus kotschoubeyanus* had the fewest pollinated stigmas across all treatments (75.84 ± 12.76, mean ± SE %); in contrast, *A. trigonus* had the most pollinated stigmas across treatments (98.77 ± 14.52 mean ± SE %). Across species, the natural pollination treatment yielded the most pistils with pollen tubes in the ovaries (76.22 ± 9.66 mean ± SE %), followed by cross-pollination (59.51 ± 9.79 mean SE %), and finally self-pollination (15.32 ± 4.54, mean ± SE %) (Fig. 3I–L). This general trend among pollination treatments was maintained within each species: the number of pollinated stigmas was similar among treatments, but the incidence of pollen tubes diminished in the lower parts of the style and in the ovary, particularly for the self-pollination treatment (Fig. 4). Although the presence of the pollen tubes
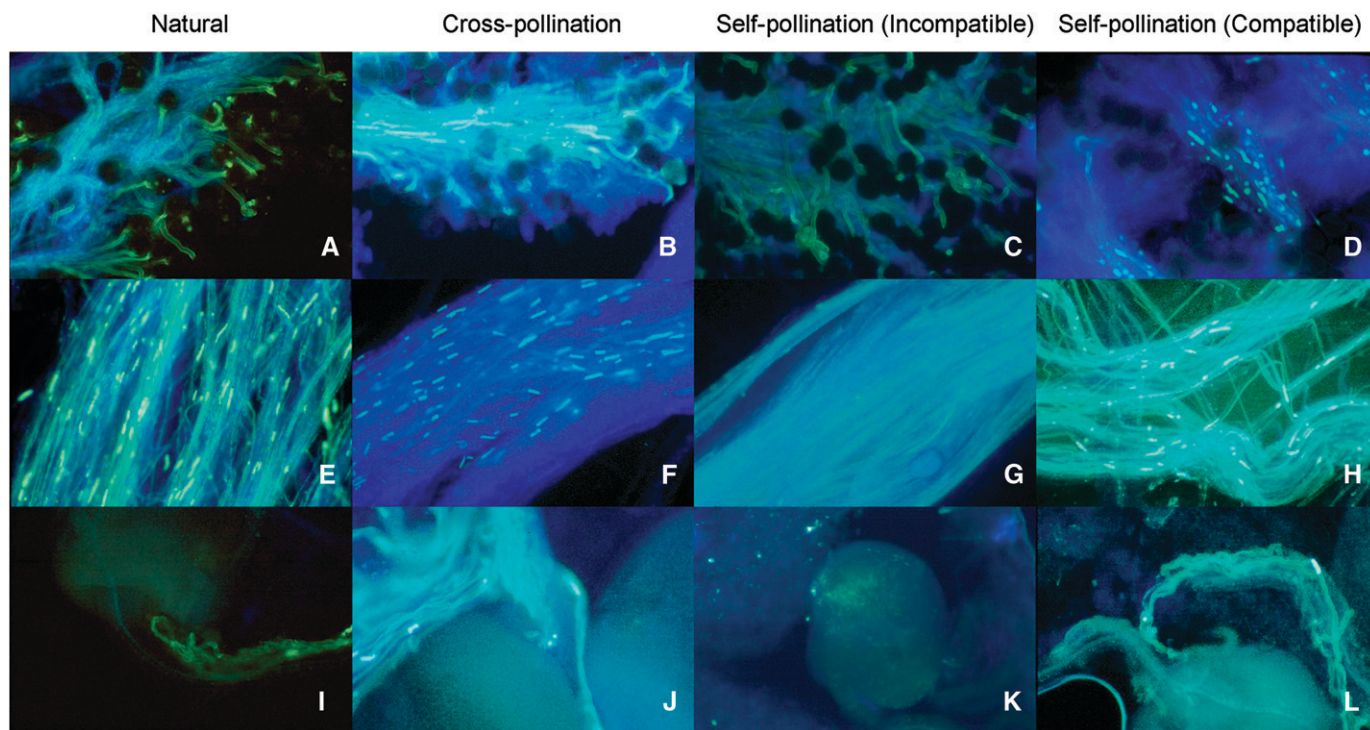
Fig. 3.   Fluorescence microscopy of pollen tube growth in squash mounts of pistils of *Ariocarpus* species after pollination treatments and aniline blue staining. Pollen germinated on stigmas after natural, cross- and self-pollinations (A–D). In natural and cross-pollinations, numerous pollen tubes grew through the style (E–F) to reach ovaries and penetrate ovules (I–J). After self-pollination, two responses can occur: (G) self-incompatibility, most pistils reject pollen tubes in the style and (K) do not reach ovaries; or (L) self-compatibility, where a small fraction of pistils permit pollen tubes to reach ovaries, similar to natural and cross-pollination treatments. (A) *A. kotschoubeyanus*, (B) *A. trigonus*, (C) *A. kotschoubeyanus*, (D) *A. trigonus*, (E) *A. retusus*, (F) *A. trigonus*, (G) *A. agavoides*, (H) *A. kotschoubeyanus*, (I) *A. kotschoubeyanus*, (J) *A. agavoides*, (K) *A. agavoides*, and (L) *A. kotschoubeyanus*.
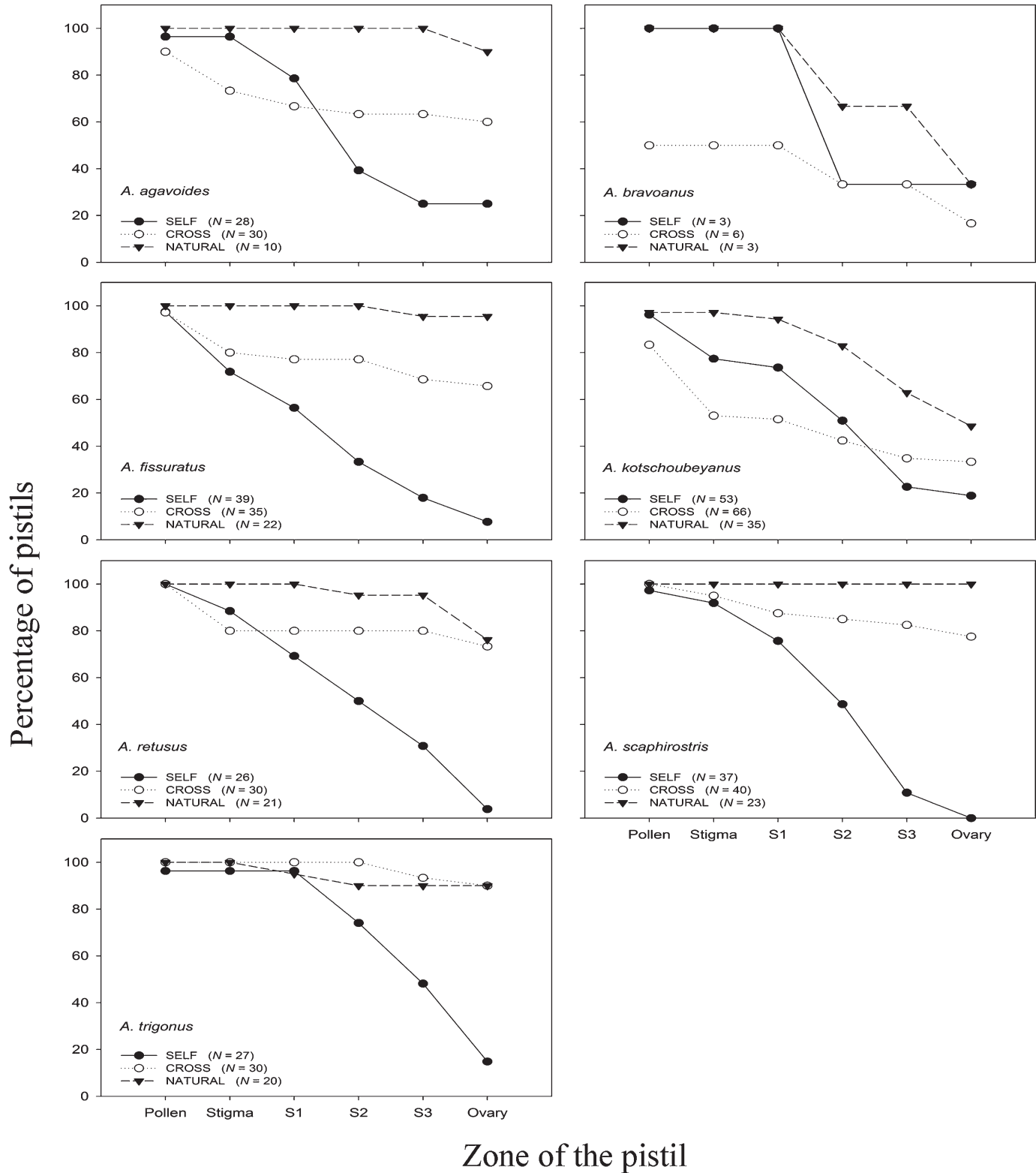
Fig. 4.   Percentage of various zones within pistils that had pollen tubes after three pollination treatments in the species of *Ariocarpus*. Pollen = presence of pollen grains in the stigma, either germinating or not. Values in parentheses are sample sizes (number of flowers) per treatment.

diminished with decreasing distance to the ovary, natural pollinations generally yielded the highest incidence of pollen tubes throughout the stylar transmitting tissue (Fig. 4).

In *A. trigonus*, pollen tube growth from the stigma to the stylar zone 1 exhibited similar success across all three treatments; however, in the selfing treatment, the presence of pollen tubes

in the pistils diminished drastically between stylar zone 2 and the ovary. Number of pistils with pollen tubes reaching the ovary was not significantly different between cross- and natural pollination, but both were significantly higher than the number of pistils with pollen tubes in the ovary following selfing (χ^2 = 20.02, df = 2, P < 0.0001) (Fig. 4).

In *A. fissuratus*, *A. retusus*, and *A. scaphirostris*, both cross- and self-pollinations had slightly fewer pollinated pistils than after natural pollination. The frequency of pollen tubes diminished drastically in selfed pistils so that the frequency of pollen tubes in the ovary was the lowest in this treatment (*A. fissuratus*, χ^2 = 48.96, df = 2, P < 0.0001; *A. scaphirostris*, χ^2 = 45.64, df = 2, P < 0.0001; *A. retusus*, χ^2 = 61.96, df = 2, P < 0.0001) (Table 1).

In four species, there was a significant interaction between stylar zone and pollination treatment: the frequency of pollen tube occurrence was found less often in the upper parts of the style after cross-pollination compared with the other treatments, but this tendency changed in the lower stylar zones such that fewer styles in self-pollinated ovaries had pollen tubes (*A. agavoides*, χ^2 = 26.64, df = 10, P = 0.003; *A. kotschoubeyanus*, χ^2 = 27.44, df = 10, P = 0.0022; *A. scaphirostris*, χ^2 = 28.48, df = 10, P = 0.0015; *A. retusus*, χ^2 = 25.91, df = 10, P = 0.0039) (Fig. 4). In *A. bravoanus*, there were no significant differences in styles with pollen tubes due to pollination treatment alone (χ^2 = 5.29, df = 2, P = 0.0709), although differences were found among the pistil zones (χ^2 = 25.44, df = 5, P < 0.0001).

Fruit set patterns were predicted by pollen tube growth: natural and hand cross-pollinations were more successful than self-pollination. Comparisons between pollen tube observations and fruit set indicated that in most cases percentages of ovaries with pollen tubes were higher than fruit set (positive values) (Table 1). Those cases in which fruit set was higher than pollen tube incidence in the ovary resulted in negative numbers. For instance, in *A. kotschoubeyanus*, all three treatments had negative values; in *A. scaphirostris*, negative values were due to 0% of the ovaries having pollen tubes, but fruit set in self-pollination was 2%. Pooled across species, mean values of absolute differences of comparisons between pollen tube production and fruit set were higher after natural pollination (27.64 ± 5.55, mean ± SE %) and cross-pollination (22.87 ± 5.87, mean ± SE %), and lower after self-pollination (10.52 ± 4.59, mean ± SE %). Number of ovules differed significantly among species (χ^2 = 389.3, df = 6, P < 0.0001), and seed to ovule ratio also varied widely (Table 2). Seed to ovule ratio following selfing was zero in *A. bravoanus* because no fruits were set in this treatment.

DISCUSSION

Our results showed that self-incompatibility occurred in natural populations of *Ariocarpus*. As previously described for Cactaceae, in most *Ariocarpus* flowers, self-pollen was inhibited in the various zones of the style, indicating gametophytic SI (Boyle et al., 1994; Boyle, 1997; Boyle and Idnurm, 2001; Mandujano et al., 2010). In six species, some genotypes were self-compatible because pollen tubes were observed in the ovaries, and in one species we did not find pollen tubes in the ovary, but there was a 2% fruit set for self-pollination. In *Mammillaria grahamii* (Bowers, 2002) and *Ferocactus* spp. (McIntosh, 2005), a higher success of outcrossed fruits and low success after manual selfing was also found.

The small proportion of pistils with nongerminating pollen in the stigma could be due to a small fraction of nonviable pollen, although pollen viability in *Ariocarpus* was previously shown to be high for all seven species (from 80.7 ± 5.36 in *A. retusus* to 95.5 ± 1.01 in *A. trigonus*, Martínez-Peralta et al., in press), and low male fertility is an unlikely cause of reduced pollen germination. However, as hand pollinations (both self and cross) were not as successful as natural pollinations in the percentage of pollinated pistils, it is possible that manipulation of both flowers and pollen may decrease the germinability of pollen grains on the receiving pistils.

The incidence of pollen tubes reaching the ovary was highest after natural pollination, and for some species, was slightly lower in cross-pollinated pistils; however, the significantly lower success of self-pollination supports previous evidence that *Ariocarpus* species are predominantly outcrossers due to self-incompatibility (Pimienta and del Castillo, 2002; McIntosh, 2005; Martínez-Peralta and Mandujano, 2011; Fuentes-Mayo, 2012; Martínez-Peralta et al., in press). Analyses showed that success among treatments varied mostly in the lower parts of the style and in the ovary, which indicates stylar inhibition of pollen tubes. Pollen tubes were mostly inhibited in the upper middle of the style in *A. agavoides* and *A. bravoanus*; however, rejection was more uniform within the style of the remaining five species. Despite these differences, pollen germination on the stigma and its rejection within the style is typical of species with gametophytic self-incompatibility in Cactaceae (Boyle et al., 1994; Boyle, 1997; Boyle and Idnurm, 2001) and angiosperms in general (de Nettancourt, 1977).

Based on the incidence of pollen tubes in the ovary of self-pollinated pistils, coupled with fruit set after self-pollination, some individuals apparently produce selfed fruits. Of the seven species, *A. kotschoubeyanus* and *A. agavoides* had the highest proportion of selfed fruits, and the highest percentage of pollen tubes in the

TABLE 1. Percentage of ovaries with pollen tubes (PT in ovaries) and of fruit set in seven *Ariocarpus* species after various hand-pollination treatments.

Species	Natural			Cross			Self		
	PT in ovaries (N)	Fruit set (N)	Difference	PT in ovaries (N)	Fruit set (N)	Difference	PT in ovaries (N)	Fruit set (N)	Difference
A. agavoides	90 (10)	41.7 (12)	48.33	60 (30)	54.5 (33)	5.45	25 (28)	6.9 (29)	18.1
A. bravoanus	33.3 (3)	16.7 (6)	16.67	16.7 (6)	53.8 (13)	−37.18	33.3 (3)	0 (5)	33.33
A. fissuratus	95.5 (22)	53.3 (182)	42.16	65.7 (35)	20.5 (44)	45.26	7.7 (39)	1.5 (67)	6.2
A. kotschoubeyanus	48.6 (35)	82.4 (17)	−33.78	33.3 (66)	46.3 (150)	−12.94	18.9 (53)	19 (124)	−0.1
A. retusus	76.2 (21)	69.8 (53)	6.38	73.3 (30)	47.8 (69)	25.51	3.8 (26)	4.3 (69)	−0.5
A. scaphirostris	100 (23)	78.1 (32)	21.88	77.5 (40)	72 (50)	5.50	0 (37)	2 (50)	−2
A. trigonus	90 (20)	65.7 (35)	24.29	90 (30)	61.8 (34)	28.24	18.5 (27)	5.1 (39)	13.39

Notes: Difference refers to % ovules with pollen tubes − % fruit set. N = sample sizes for each trial.

TABLE 2.　Ovules per flower, seed set, and seed to ovule ratio in the seven species of *Ariocarpus*.

Species	Ovules (*N* flowers)	Seeds			Seed/ovule		
		Natural (*N* fruits)	Cross (*N* fruits)	Self (*N* fruits)	Natural	Cross	Self
A. agavoides	49.6 a (35)	45.6 (10)	28.15 (18)	20.5 (3)	0.92	0.57	0.41
A. bravoanus	68.52 b (21)	7.92 (13)	13.5 (6)	0	0.12	0.20	0
A. fissuratus	93.38 c (33)	69.79 (58)	41.57 (7)	68 (1)	0.75	0.45	0.73
A. kotschoubeyanus	45.83 a (36)	33.57 (7)	26.42 (24)	24.86 (7)	0.73	0.58	0.54
A. retusus	122.61 d (38)	43.41 (29)	44.39 (28)	66 (2)	0.35	0.36	0.54
A. scaphirostris	26.52 e (27)	23.06 (17)	16.83 (24)	11 (1)	0.87	0.63	0.41
A. trigonus	115.78 d (25)	115.13 (23)	70.21 (19)	70.21 (2)	0.99	0.61	0.25

ovary. *Ariocarpus bravoanus* also showed a high percentage of pollen tubes in the ovary. *Ariocarpus fissuratus*, *A. retusus*, and *A. trigonus* showed the lowest percentages of pistils with pollen tubes in the ovary, but those percentages could reflect the reduced occurrence of compatible genotypes in their populations. We found strict self-incompatibility in *A. scaphirostris*; ovaries from selfed pistils completely lacked pollen tubes. Therefore, our results indicate that in six *Ariocarpus* species, most of the individuals are self-incompatible, but some genotypes are capable of self-pollination, a pattern described as pseudo- or partial SI (PSI) (Levin, 1996; Nielsen et al., 2003; Ferrer et al., 2009), which represents a modified expression of SI and could represent a transition from strict self-incompatible to self-compatible populations. Mutations that lead to self-compatibility seem to be relatively frequent in natural populations (Good-Ávila et al., 2008) and are frequently related to environmental (e.g., increased heat, light conditions) or endogenous factors (e.g., floral age, available resources) (Bittencourt and Semir, 2005; Good-Ávila et al., 2008). From an evolutionary point of view, partial SI may emerge in environments with pollen or mate limitation (e.g., Ferrer et al., 2009), as low mate availability may reduce seed set and increase the risk of local extinction, so self-compatible genotypes are selected to achieve reproductive assurance (Schoen et al., 1996). To explore possible causes of occurrence of self-compatible individuals in natural populations of *Ariocarpus*, further studies should address issues such as effective population size, relatedness among individuals, and pollinator limitation. For instance, seed set in *A. kotschoubeyanus* is pollen limited (Martínez-Peralta et al., in press), which could promote the emergence of partial SI in this species, which had the highest incidence of selfed pollen tubes in the ovary. Further studies in *Ariocarpus* must take into account the timing of rejection or the presence of abortion in the ovary of selfed flowers to rule out the possibility of processes such as late-acting or postzygotic SI (Bittencourt and Semir, 2005). For instance, inhibition of self-pollen in *Hylocereus* (Cactaceae) occurs in the ovary (Lichtenzveig et al., 2000) and is regarded as late-acting SI. Other studies reported that ovule penetration in selfed pistils is related to floral age, but pistils are finally aborted (Bittencourt and Semir, 2005).

Most of the naturally pollinated pistils had pollen on the stigma, suggesting that pollinators are effective in transporting pollen grains. The exception was *A. kotschoubeyanus*, with the lowest percentage of naturally pollinated pistils and evidence of pollen limitation in seed set (Martínez-Peralta et al., in press). According to the comparisons between pollen tube production and fruit set, and seed to ovule ratios, the presence of pollen tubes in the ovary does not necessarily imply the development of fruits and seeds. In all seven species, there were differences between the percentage of naturally pollinated pistils and fruit set, particularly in *A. agavoides* and *A. fissuratus*. Cross- and self-pollinations also yielded differences, and these differences were higher in the cross-pollination treatment across species. In

A. kotschoubeyanus, the higher success of fruit set relative to the presence of pollen tubes in the ovary for all treatments could be explained by a longer time needed for the pollen tubes to reach ovaries. Flowers that were used to measure fruit set had more time to allow pollen tube growth, while flowers were collected for microscopy 48 h after the last pollination. Seed to ovule ratio of natural pollination was above 70% for five species, which is higher than the mean value reported for perennials (50%, Wiens, 1984); for two species, this ratio was lower (0.12 for *A. bravoanus* and 0.35 for *A. retusus*). Seed to ovule ratio in cross- and self-pollination treatments varied widely, but there is no pattern among treatments or species. The lower fruit set relative to the number of pollinated flowers, and low seed to ovule ratios, have been attributed to early inbreeding depression (genetic load, sensu Wiens et al., 1987), a threshold of fertilized ovules to elicit fruit formation, or resource limitation (Stephenson, 1981). For example, in the fully SI *Opuntia microdasys*, fruit set of outcrossed and naturally pollinated flowers increased 11% after watering and fertilization, but selfed flowers failed to set fruit regardless of differences in resource availability (Piña et al., 2007). Experimental designs involving the manipulation of maternal resources, pollen load on the stigma, and observations of early developing embryos could account for clarify the effect of these processes.

Outcrossing within Cactaceae seems to be widespread (review: Mandujano et al., 2010), but there is a paucity of detailed studies of self-incompatibility in natural populations, where variation in the strength of systems governing outcrossing can occur. Pollen tube growth results showed the presence of self-incompatibility in the seven species of the genus *Ariocarpus*, with six species being partially and one completely SI. Stylar rejection of pollen tubes indicates GSI, as previously reported for some species of the family. The presence of SI limits the number of compatible mates and can diminish opportunities for sexual reproduction in populations when floral display is unfavorable or limited. The production of selfed offspring via compatible genotypes could represent an evolutionary response of SI systems (Stephenson et al., 2000) in particular for *A. agavoides* and *A. kotschoubeyanus*, suggesting that SI systems are not static in natural populations of *Ariocarpus*.

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