

Sexual compatibility between eight *Nolana* L.f. (Solanaceae) species from Peru and Chile

Amy C. Douglas · Rosanna Freyre

Received: 26 June 2014 / Accepted: 24 May 2015 / Published online: 17 June 2015
© Springer Science+Business Media Dordrecht 2015

Abstract Artificial hybridizations (self- intra- and interspecific) were performed within and between eight species of *Nolana* (*N. adansonii*, *N. aticoana*, *N. elegans*, *N. humifusa*, *N. ivaniana*, *N. laxa*, *N. plicata* and *N. rupicola*). Plant material originated from three fog oases (“lomas”) locations in the Peruvian desert in Peru and two “lomas” locations in the Atacama desert in Chile. Self-pollinations and all possible crosses, including reciprocals, were made between three and five accessions of each species. Crosses were made in replicates of five (or ten in self-pollinations), totaling 310 self-pollinations, 480 intraspecific pollinations, and 2535 interspecific pollinations. Species were generally self-incompatible, with only one accession of *N. adansonii* and one accession of *N. ivaniana* exhibiting limited self-fertility as measured by fruit set. Intraspecific compatibility appears to be high with most crosses resulting in greater than 80 % fruit set. Success of interspecific hybridization was initially analyzed based on fruit set, mericarps per fruit and mericarp size, and confirmed by seed counts through

X-ray analyses of mericarps, and seed germination. Differences in all these factors were found within seed parent species in response to hybridization with different pollen parent species. Reduction in interspecific fertility was generally seen as lower fruiting success and smaller mericarp size when compared to intraspecific hybridization, while the number of mericarps per fruit remained constant. Seed set was estimated by X-ray analysis of mericarps, revealing differences in seed counts between crosses. Mericarps were sown producing hybrid seedlings from 22 unidirectional species pairs. Previous to these studies, sexual compatibility was known to exist only between *N. paradoxa* and four other species. The results of these studies are a significant contribution to the current knowledge of sexual compatibility within *Nolana* and are important for future breeding efforts.

Keywords Artificial hybridization · Interspecific · Intraspecific · Mericarp

Introduction

Nolana L.f. was previously considered a unique family (Nolanaceae) due to its 5-carpelled gyonecium, but is now included as a genus within the Solanaceae based on chloroplast DNA analysis (Olmstead and Palmer 1992). *Nolana* is the fourth largest genus in the Solanaceae, with 89 species primarily distributed in the Atacama and Peruvian deserts, a few species in the

A. C. Douglas
University of New Hampshire Cooperative Extension,
Merrimack County, 315 Daniel Webster Highway,
Boscawen, NH 03303, USA

R. Freyre (✉)
Environmental Horticulture Department, Institute of Food
and Agricultural Sciences, University of Florida,
P.O. Box 110670, Gainesville, FL 32611, USA
e-mail: rfreyre@ufl.edu

inland regions of the Andes, and one species endemic to the Galapagos Islands (Tu et al. 2008). In the deserts, *Nolana* species are found in isolated patches of vegetation (“lomas”) which are dependent on fog conditions during winter months (June–September). These areas flourish during El Niño years when the “lomas” experience unusually high rainfall and humidity (Dillon 2005). A “loma” area may support a single *Nolana* species or up to 11 species growing sympatrically (Dillon et al. 2009). Most species are narrow endemics, but few have wide distributions.

Nolana species range from herbaceous annuals to moderately woody perennial shrubs (Tago-Nakawaza and Dillon 1999). Most species display showy flowers borne singly in leaf axils. Flowers are tubular-salverform to campanulate, infundibular, or rotate and range in size (1–8 cm diameter) and in color (blues, purples, pinks, and whites) (Freyre et al. 2005). The fruit of *Nolana*, the mericarp, is a unique derived character in the Solanaceae (Knapp 2002). Fruit morphology varies by species, and based on observations of field-collected open pollinated fruits they consist of two to 30 highly sclerified mericarps, which are unilocellate or plurilocellate, and contain one to seven individual seeds each (Tago-Nakawaza and Dillon 1999). Each seed within a mericarp has an associated funicular plug which is displaced upon germination providing a canal through which the seedling exits the mericarp (Bondeson 1986). Due to the plurilocellate characteristic of some mericarps, multiple seedlings may germinate from a single mericarp.

Cytological studies of *Nolana* are few, but suggest a chromosome count of $x = 12$. $2n = 2x = 24$ has been reported for *N. humifusa* (as *N. prostrata*) (Campin 1925; Datta 1933), *N. paradoxa* (as *N. atriplicifolia*) (Campin 1925; Datta 1933; di Fulvio 1969), and *N. rostrata* (di Fulvio 1984). Reports of sexual compatibility within *Nolana* are very limited. Self-compatibility in *N. paradoxa* and in *N. humifusa* was reported, as well as successful hybridization between *N. paradoxa* (as *N. atriplicifolia*) and *N. humifusa* (as *N. prostrata*), both artificial and spontaneous in cultivation (Saunders 1934). Cultivated *N. paradoxa* ‘Bluebird’ was successfully hybridized using wild *N. elegans*, *N. rupicola*, and *N. aplocaryoides* as male parents (Freyre et al. 2005).

Included in this study are six Peruvian species collected from four “lomas” areas (*N. adansonii*, *N. aticoana*, *N. humifusa*, *N. ivaniana*, *N. laxa*, and *N.*

plicata) and two Chilean species collected in two “lomas” areas (*N. elegans* and *N. rupicola*). Recently, the phylogeny of *Nolana* was constructed from sequences of four plastid markers and the nuclear LEAFY second intron. Results indicate that *N. humifusa*, *N. aticoana* and *N. adansonii* are grouped in one clade, *N. laxa*, *N. plicata* and *N. ivaniana* in a second clade, and *N. elegans* and *N. rupicola* in a third clade (Tu et al. 2008; Dillon et al. 2009). Floral development, stigma receptivity and pollen viability of these eight *Nolana* species was reported in a previous study (Douglas and Freyre 2010). Additionally, these eight *Nolana* species were included among 11 species in a study of reproductive isolation, which concluded that postzygotic isolation (lack of fruit set, mericarp size and seed set) was generally stronger and faster evolving than prezygotic isolation (pollen-pistil interactions) (Jewell et al. 2012).

In this study, sexual compatibility (including self-, intra-, and interspecific-compatibility) within eight *Nolana* species was investigated. Self-pollinations, pollinations between accessions within each species, and pollinations between all possible combinations of species were performed by manual pollination. Sexual compatibility was evaluated based on fruiting success, mericarp and seed characteristics, and seed germination. These studies are a significant contribution to the current knowledge of sexual compatibility within *Nolana* and are important for future breeding efforts.

Materials and methods

Plant material

Plant material included three to five accessions from each of eight *Nolana* species (*N. adansonii*, *N. aticoana*, *N. elegans*, *N. humifusa*, *N. ivaniana*, *N. laxa*, *N. plicata*, and *N. rupicola*) which were vegetatively propagated and grown to maturity (Table 1). One plant was used per accession. Each accession constituted a distinct genotype, which was either vegetatively propagated from a plant found in the wild, or as a seedling grown from (open pollinated) mericarps collected from a plant found in the wild. Herbarium vouchers are housed at the Hodgdon Herbarium, University of New Hampshire, and at the Field Museum of Natural History, Chicago, IL. Photos

Table 1 Species, accession codes and habit of *Nolana* plants used in sexual compatibility studies

Species	Accession code ^y	Habit ^x
<i>N. humifusa</i>	H28, Hu1-2, Hu9-4	Herbaceous annual
<i>N. laxa</i>	La1-2, La1-4, La1-5 ^z , La3-1, La3-2 ^z	Erect herbaceous perennial
<i>N. plicata</i>	P5, P7, P11	Herbaceous perennial
<i>N. aticoana</i>	A2, A3, A13	Herbaceous perennial
<i>N. adansonii</i>	Ad2-2, Ad2-3 ^z , Ad4-1, Ad4-11, Ad4-14	Erect herbaceous perennial
<i>N. ivaniana</i>	Iv2-1, Iv2-2 ^z , Iv2-3, Iv2-5	Erect herbaceous annual
<i>N. elegans</i>	Ele1 ^z , Ele2, Ele3 ^z , 051-3, 051-5	Procumbent herbaceous annual
<i>N. rupicola</i>	Rup1, Rup2, Rup3	Herbaceous perennial

^x Adapted from Tago-Nakazawa and Dillon (1999)

^y Herbarium vouchers housed at UNH Hodgdon Herbarium, Durham, NH and at the Field Museum of Natural History, Chicago, IL

^z Individuals used in intraspecific hybridizations only. All others used in both intraspecific and interspecific hybridizations

of representative accessions of each species have been shown elsewhere (Douglas and Freyre 2010).

Species were selected to represent a range of geographic areas to which *Nolana* are endemic. Accessions were collected from three areas in Peru and one area in Chile, with two species being collected from each area (Fig. 1). *Nolana humifusa* and *N. laxa* are endemic to the northern ranges of *Nolana* habitat. *Nolana humifusa* was collected from coastal Lomas de Pachacamac, Lima, Peru and *N. laxa* from hillsides of Los Condores, Lima, Peru. The two locations are separated by approximately 50 km. *Nolana humifusa* is an herbaceous annual with delicate trailing foliage and small pale blue flowers (2–3 cm diameter) marked with conspicuous dark purple veins. *Nolana laxa* is an erect herbaceous perennial with narrow slightly pubescent foliage and larger deeply funnelform purple flowers (3–4 cm diameter). *Nolana plicata* and *N. aticoana* were collected approximately 500 km south of Lima at Lomas de Atiquipa, Arequipa, Peru. The species were found growing separately with *N. aticoana* growing at a higher elevation (450–480 masl) than *N. plicata* (400 masl). Both species are herbaceous perennials. Flowers of *N. plicata* are a medium-pale blue in color (4–5 cm diameter) and foliage is trailing and pubescent. *Nolana aticoana* exhibits smaller medium-blue flowers (3–4 cm diameter) and glossy trailing foliage. *Nolana adansonii* and *N. ivaniana* were collected approximately 300 km south of Atiquipa, on the seashore of Catarindo Beach, Arequipa, Peru. *Nolana adansonii* was found growing on the sand and hills in close proximity to the sea while *N. ivaniana* was found

approximately 30 m inland. Both species are erect herbaceous perennials. *Nolana adansonii* is slightly woody with small cordate foliage. Flowers are small, purple, and deeply funnelform (2–3 cm diameter). *Nolana ivaniana* exhibits pale, narrow, pubescent foliage and small, pale blue flowers (2.5–3.5 cm diameter). *Nolana elegans* and *N. rupicola* are endemic to the southern range of *Nolana* habitat. *Nolana elegans* was collected at Cerro Perales, Chile, approximately 1600 km south of Arequipa, Peru. This species is an herbaceous annual with large, bright blue flowers (4–5 cm diameter) and procumbent pubescent foliage. *Nolana rupicola* was collected approximately 70 km south of *N. elegans* at Las Lomitas, Chile. *Nolana rupicola* is an herbaceous perennial with large blue flowers (5–6 cm diameter). Foliage is procumbent and pubescent with a central rosette.

Plant material was maintained in an insect-exclusion double-poly hoop house located at the University of New Hampshire Woodman Farm, Durham, NH. Plants were grown in 2 L pots with soilless media (Sunshine LA4 aggregate mix; SunGro Horticulture Inc., Bellevue, WA). Fertilization was constant with a 20N–4.3P–16.7K fertilizer at 150 mg L⁻¹ N. Average air temperature was recorded for each stage of the study with a HOBO temperature logger (H08-001-02; Onset Corp., Bourne, MA). Average daily air temperatures were 21.1 and 25.8 °C for the periods of 23 Dec. 2004 through 13 Jan. 2005, and 7 July 2005 through 15 Aug. 2005, respectively. Plants were grown under natural day length and light intensity and no difference was noted in plant flowering or health in different seasons.

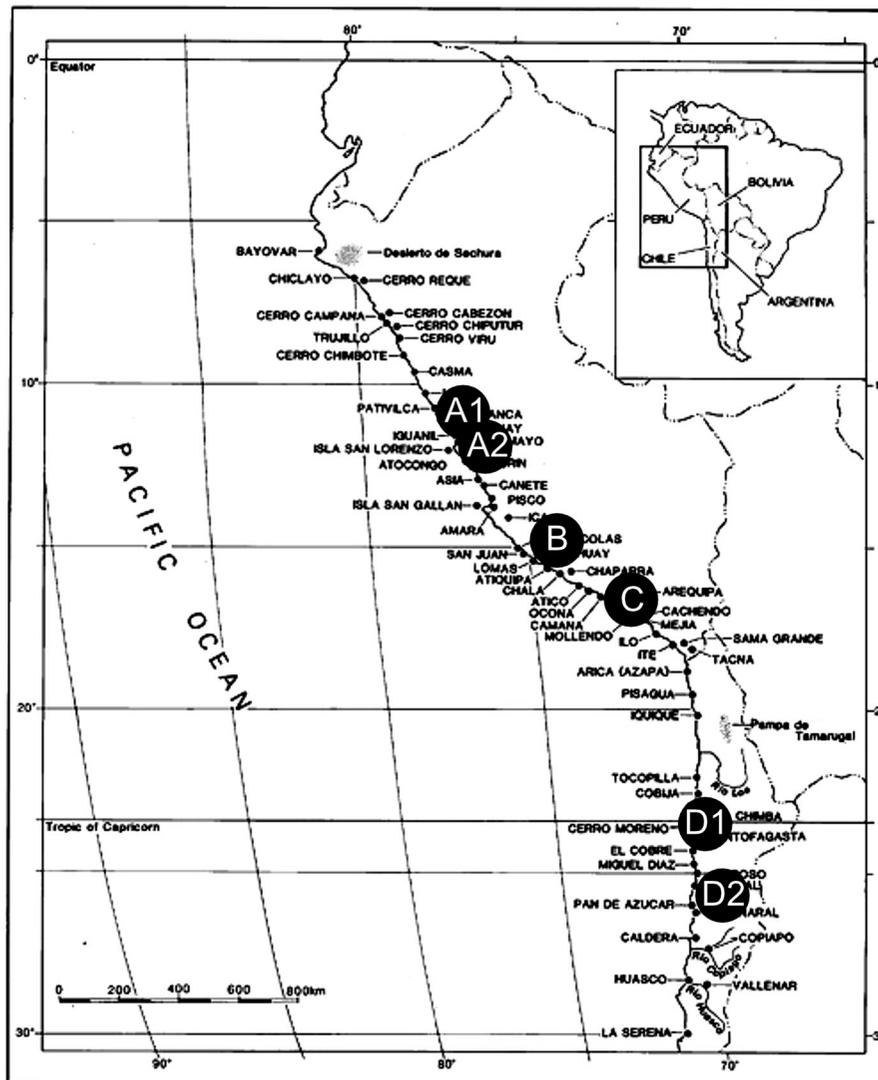


Fig. 1 Collection locations of plant material included in sexual compatibility studies. Labeled locations indicate “lomas” areas, where *Nolana* species are found seasonally when fog and rain are available. (A1, A2) collection sites of *N. humifusa* and *N. laxa*.

(B) *N. plicata* and *N. aticoana*. (C) *N. adansonii* and *N. ivaniana*. (D1, D2) *N. elegans* and *N. rupicola*. Map adapted from Dillon et al. 2003

Self-pollinations

Ten flowers per accession were emasculated at a late bud stage prior to anthesis and pollen dehiscence. Dehiscent pollen from recently opened flowers on the same plant was manually applied by gently touching anthers to stigmas of emasculated buds. Self-pollinations were performed between Dec. 2004 and Feb 2005.

Intraspecific hybridization

Manual pollination between three and five accessions within each of eight *Nolana* species was performed between Jan. 2005 and March 2005. Five flowers were pollinated for each possible cross within each species. Pollinations were performed on partially open or recently opened flowers by touching anthers of recently opened flowers of the pollen parent to stigmas of seed parent.

Interspecific hybridization

Three accessions from each of eight *Nolana* species were selected for use in the interspecific compatibility study (Table 1). Due to death of one individual of *N. adansonii* from disease before completion of the hybridization scheme, a fourth accession of this species was also included. Manual pollinations were performed between Aug. 2005 and March 2006. Partially open or recently opened flowers of the seed parent were pollinated with pollen from recently opened flowers of the pollen parent. Five flowers were pollinated for each accession-cross. In total, 2535 interspecific pollinations were performed representing nine accession-crosses in each of 56 species-crosses.

Harvest and storage

Fruits from successful hybridizations were left on the parent plant to mature. Fruits were harvested when mericarps became dark and, upon gentle squeezing of the fruit, were found to be loosened from the receptacle. Harvested mericarps were stored in paper coin envelopes in a desiccator at room temperature.

Data collection

Success of fruit set was recorded for each pollination. Counts were made of the number of mericarps per fruit from five fruits per intraspecific cross and from all successful interspecific crosses. Average mericarp mass of each cross was calculated from measurement of combined mass of up to 50 mericarps per intraspecific cross or all available mericarps per interspecific cross. Average mericarp diameter of each cross was calculated from measurement of diameter of each mericarp at the widest area of the mericarp plug face on ten mericarps per cross. To obtain sufficient numbers of mericarps for size analyses, intraspecific crosses which had limited fruit set were repeated until at least five fruits were obtained.

Mericarps of each species and from each interspecific cross were examined with X-rays (Faxitron MX20 Digital X-ray at the Ornamental Plant Germplasm Center, Ohio State University, Columbus, OH) to estimate number of seeds per mericarp. Random samples of 12–286 mericarps from each cross and species were X-rayed and digital images were

recorded. Based on differences in contrast within the recorded images, counts were made of the number of presumed full seeds, and of cavities appearing empty or containing abnormally formed seed in each mericarp. Abnormal seeds were identified as those which did not appear to fully fill the cavity.

All mericarps from interspecific hybridizations were sown in Aug. 2006 in seed trays with a 2:1 peat/perlite propagation medium at a distribution of one mericarp per cell. Mericarps were germinated under natural day length with an average temperature of 22 °C and relative humidity of 73 %. Number of seedlings germinating from each mericarp was recorded. Germinated seedlings were transplanted into 15-cm pots and were grown in the greenhouse for future studies.

Results and discussion

Self-incompatibility

All species exhibited a high degree of self-incompatibility. Six species (*N. aticoana*, *N. elegans*, *N. humifusa*, *N. laxa*, *N. plicata*, and *N. rupicola*) failed to produce fruit upon self-pollination. One accession of *N. adansonii* (Ad2-3) and one accession of *N. ivaniana* (Iv2-2) exhibited limited self-compatibility, each with two out of ten pollinations resulting in fruit set. Fruits of Ad2-3 resulting from self-pollination contained fewer mericarps (average = 7.3) than those from intraspecific hybridizations involving the same seed parent (average = 15.3). Number of mericarps per fruit was similar between fruits resulting from self-versus intraspecific pollination of Iv2-2, however, mericarps resulting from self-pollinations were smaller in mass and diameter (self: average mass: 3.3 mg, average diameter: 1.4 mm versus intra: average mass: 8.3 mg, average diameter: 2.4 mm), suggesting reduced seed set. Results show that while some self-compatibility exists within *Nolana*, species are generally self-incompatible. Gametophytic self-incompatibility is a common trait within Solanaceae (de Nattencourt 1977). Based on these results, it was determined that emasculation prior to anther dehiscence was unnecessary in all but two of the studied accessions when used in our subsequent studies of intra- and interspecific sexual compatibility. This conclusion was supported by the facts that plants were

housed in a pollinator-free greenhouse and that observed anther and stigma morphology does not facilitate unassisted self-pollination.

Intraspecific sexual compatibility

Differences were evident between species in number of mericarps per fruit and in mericarp size. *N. rupicola* had the highest number of mericarps per fruit with an average of 18.3, and *N. laxa* had the fewest with an average of 3.8. Average mericarp mass ranged between species from 0.7 mg per mericarp in *N. adansonii* to 35 mg per mericarp in *N. plicata*. Average mericarp diameter ranged between species from 1.2 mm in *N. adansonii* to 4.6 mm in *N. plicata*.

Using fruit set, species exhibited high intraspecific sexual compatibility. All but 23 out of 96 attempted crosses resulted in at least 80 % fruit set. A few intraspecific crosses exhibited incompatibility (failure of fruit set) or limited compatibility (20–60 % fruiting success, data not shown). Average intraspecific fruiting success, pollination success, mericarps per fruit, and mericarp mass and diameter for each species is summarized (Table 2). Seven out of the eight studied species exhibited overall fruiting success greater than 80 % fruit set with intraspecific pollination. *N. ivaniana* had unusually low fruiting success at 68 %, possibly due to failing health of the plants at time of study. Germination of seed from intraspecific pollinations was not evaluated in this study. *Nolana* seed exhibits dormancy mechanisms which we have been unable to effectively overcome. Germination percentages are low and not representative of true seed viability.

Interspecific sexual compatibility

Sexual compatibility exists between species if cross-pollination between them results in production of viable hybrid seed. Determination of viable seed production has been a challenge in our studies of *Nolana*. Although pollinations frequently result in successful fruit production, verification of existence of viable seed within those fruit has not been possible in many cases. Seed germination is commonly used as a simple measure of seed viability. However, as mentioned previously, *Nolana* seed exhibit dormancy mechanisms. We have been unable to determine

whether failure of hybrid seed germination is due to seed inviability or due to seed dormancy. A second commonly used measure of seed viability is tetrazolium chemical enzyme staining. This method has also proven ineffective in *Nolana*. *Nolana* seeds are contained within highly sclerified mericarps. We were unable to excise seed from the stony mericarps without inflicting injury to the seed which causes false positive results and nullification of enzyme staining. Since we could not directly document sexual compatibility by measure of viable hybrid seed production, we measured levels of compatibility indirectly using values of fruit set, mericarps per fruit, mericarp sizes, and seeds per mericarp for each cross. By comparing the values obtained through interspecific hybridization to those obtained through intraspecific hybridizations, degrees of compatibility between species can be inferred. In some cases, we have been able to verify existence of sexual compatibility between species by germination of hybrid seed. However, germination cannot be used as a measure of degree of compatibility, because germination percentages are typically low and are unlikely representative of viable seed production.

Fruit set

Successful fruit set was used as an initial indicator of potential sexual compatibility between species. Results show that fruiting success in interspecific crosses is common in the *Nolana* species studied. Hybridization in 32 out of 56 species crosses resulted in at least one instance of successful fruit set (Table 3). Within each compatible species cross, successful fruit set occurred in a range of one to nine accession crosses out of a possible nine, while total number of fruits developed per species cross ranged from three to 43 fruits out of a possible 45.

Bilateral compatibility was seen in ten species pairs, unilateral compatibility in 12, and bilateral incompatibility in six (Table 3). Within bilaterally compatible crosses, five were between species that are in the same clade based on molecular analyses, and five between species that are in different clades (Tu et al. 2008; Dillon et al. 2009).

Differences were evident in levels of compatibility between reciprocals. For example, *N. humifusa* × *N. aticoana* was highly successful with nine out of nine

Table 2 Average fruiting, mericarps per fruit, mericarp mass and diameter for intraspecific hybridizations within eight *Nolana* species

	Average fruiting success (%)	Average mericarps per fruit	Average mericarp mass (mg)	Average mericarp diameter (mm)
<i>N. humifusa</i>	100	4.9 ± 0.3	9.1 ± 3.5	2.6 ± 0.4
<i>N. laxa</i>	91	3.8 ± 1.0	8.0 ± 2.2	2.4 ± 0.5
<i>N. plicata</i>	100	4.1 ± 1.0	35.0 ± 12.9	4.6 ± 1.0
<i>N. aticoana</i>	97	4.8 ± 0.4	25.8 ± 5.5	3.5 ± 0.5
<i>N. adansonii</i>	82	15.5 ± 2.0	0.7 ± 0.1	1.2 ± 0.2
<i>N. ivaniana</i>	68	8.4 ± 2.7	8.9 ± 3.6	2.4 ± 0.6
<i>N. elegans</i>	83	10.7 ± 5.4	6.2 ± 2.3	2.1 ± 0.4
<i>N. rupicola</i>	100	18.3 ± 1.9	9.0 ± 0.7	2.4 ± 0.5

accession crosses and 43 out of 45 individual pollinations resulting in fruit set. Meanwhile, the reciprocal, *N. aticoana* × *N. humifusa* was only minimally successful with four out of nine accession crosses and five out of 45 pollinations resulting in fruit set. Other species pairs, such as *N. elegans* and *N. rupicola* with a total of 71 out of 90 successful pollinations, were highly successful in both directions of hybridization. In contrast, *N. elegans* and *N. ivaniana* with a total of seven out of 90 successful pollinations, were only minimally successful in either direction of hybridization. Unilaterally compatible crosses were generally only moderately successful with fewer compatible accessions and lower overall fruit set than was seen in many bilaterally compatible crosses. Few species exhibited bilateral incompatibility, and in all cases included two species that are in different clades, with the exception of the cross *N. adansonii* × *N. ivaniana*. Interestingly, all six bilaterally incompatible species pairs included either *N. adansonii* or *N. rupicola* as a parent.

One possible cause of incompatibility could be if the *Nolana* species studied have different chromosome base numbers (i.e. such as in *Capsicum*, Pozzobon et al. 2006) or different ploidy levels. In other Solanaceae, differences in ploidy levels have been shown to cause both bilateral and unilateral incompatibilities resulting from imbalances in the endosperm balance number, or ratio of endosperm contributions from male and female parents (Ehlenfeldt and Hanneman 1988; Ortiz and Ehlenfeldt 1992). Other possibility is heteromorphic incompatibility due to differences in style lengths and pollen tube sizes. These studies are beyond the scope of this paper.

Mericarp characteristics

We are unable to dissect the sclerified mericarps of the *Nolana* fruit to determine how many seeds they contained, so we employed methods of indirectly evaluating mericarp contents. We used counts of mericarps per fruit and measurements of mericarp mass and diameter to assess the success of interspecific hybridizations. By comparing these values between those obtained through intraspecific hybridization, differences in hybridization success may be identified. Crossability indices were calculated representing ratios of inter- to intraspecific values for fruit set, mericarps per fruit, mericarp mass, and mericarp diameter for all compatible species combinations (Fig. 2). Crossability indices range from zero (indicating complete sexual incompatibility in a species cross) to one (indicating values obtained through interspecific hybridizations are as high, or higher, than those of intraspecific hybridizations of the seed parent species). Decreased fertility in interspecific hybridizations is apparent in some species crosses such as *N. laxa* × *N. humifusa*, with a reduction in fruiting success (fruit index: 0.37) while number of mericarps per fruit and mericarp size remains comparable to intraspecific values (indices of 0.80–0.91). Alternatively, in other species crosses such as *N. plicata* × *N. laxa*, apparent reduced fertility is displayed as a decrease in mericarp size with a mass index of 0.30 and a diameter index of 0.53, while number of fruits and mericarps per fruit remains unchanged (indices of 0.90 and 1.00 respectively). Indications of reduced fertility range between these two extremes in other species crosses.

Collective consideration of all factors provides a more thorough indication of reduced fertility than does

Table 3 Fruiting success in interspecific hybridizations of *Nolana*

Bilaterally compatible	Unilaterally compatible			Bilaterally incompatible			
	Crosses with fruit set ^y	Total fruits developed	Crosses with fruit set	Total fruits developed	Crosses with fruit set	Total fruits developed	
<i>N. elegans</i> × <i>N. rupicola</i> ^z	9	36	<i>N. humifusa</i> × <i>N. ivaniana</i>	8	35	<i>N. adansonii</i> × <i>N. ivaniana</i>	0
<i>N. rupicola</i> × <i>N. elegans</i>	9	35	<i>N. ivaniana</i> × <i>N. humifusa</i>	0		<i>N. ivaniana</i> × <i>N. adansonii</i>	0
<i>N. plicata</i> × <i>N. aticoana</i>	9	39	<i>N. adansonii</i> × <i>N. plicata</i>	8	19	<i>N. adansonii</i> × <i>N. elegans</i>	0
<i>N. aticoana</i> × <i>N. plicata</i>	8	37	<i>N. plicata</i> × <i>N. adansonii</i>	0		<i>N. elegans</i> × <i>N. adansonii</i>	0
<i>N. ivaniana</i> × <i>N. laxa</i>	9	34	<i>N. elegans</i> × <i>N. aticoana</i>	7	17	<i>N. adansonii</i> × <i>N. rupicola</i>	0
<i>N. laxa</i> × <i>N. ivaniana</i>	8	34	<i>N. aticoana</i> × <i>N. elegans</i>	0		<i>N. rupicola</i> × <i>N. adansonii</i>	0
<i>N. humifusa</i> × <i>N. laxa</i>	9	38	<i>N. elegans</i> × <i>N. plicata</i>	7	13	<i>N. adansonii</i> × <i>N. laxa</i>	0
<i>N. laxa</i> × <i>N. humifusa</i>	5	15	<i>N. plicata</i> × <i>N. elegans</i>	0		<i>N. laxa</i> × <i>N. adansonii</i>	0
<i>N. humifusa</i> × <i>N. aticoana</i>	9	43	<i>N. ivaniana</i> × <i>N. plicata</i>	5	19	<i>N. aticoana</i> × <i>N. rupicola</i>	0
<i>N. aticoana</i> × <i>N. humifusa</i>	4	5	<i>N. plicata</i> × <i>N. ivaniana</i>	0		<i>N. rupicola</i> × <i>N. aticoana</i>	0
<i>N. humifusa</i> × <i>N. plicata</i>	9	39	<i>N. ivaniana</i> × <i>N. rupicola</i>	5	19	<i>N. plicata</i> × <i>N. rupicola</i>	0
<i>N. plicata</i> × <i>N. humifusa</i>	2	4	<i>N. rupicola</i> × <i>N. ivaniana</i>	0		<i>N. rupicola</i> × <i>N. plicata</i>	0
<i>N. laxa</i> × <i>N. plicata</i>	8	32	<i>N. elegans</i> × <i>N. laxa</i>	5	18		
<i>N. plicata</i> × <i>N. laxa</i>	3	4	<i>N. laxa</i> × <i>N. elegans</i>	0			
<i>N. aticoana</i> × <i>N. laxa</i>	5	18	<i>N. humifusa</i> × <i>N. adansonii</i>	3	14		
<i>N. laxa</i> × <i>N. aticoana</i>	3	9	<i>N. adansonii</i> × <i>N. humifusa</i>	0			
<i>N. adansonii</i> × <i>N. aticoana</i>	4	10	<i>N. humifusa</i> × <i>N. rupicola</i>	3	14		
<i>N. aticoana</i> × <i>N. adansonii</i>	3	11	<i>N. rupicola</i> × <i>N. humifusa</i>	0			
<i>N. elegans</i> × <i>N. ivaniana</i>	1	3	<i>N. ivaniana</i> × <i>N. aticoana</i>	3	8		
<i>N. ivaniana</i> × <i>N. elegans</i>	1	4	<i>N. aticoana</i> × <i>N. ivaniana</i>	0			
			<i>N. laxa</i> × <i>N. rupicola</i>	3	6		
			<i>N. rupicola</i> × <i>N. laxa</i>	0			
			<i>N. humifusa</i> × <i>N. elegans</i>	2	3		
			<i>N. elegans</i> × <i>N. humifusa</i>	0			

^y Hybridizations were performed between eight species, three accessions per species. Accessions were cross-pollinated in every possible combination including reciprocals. Five pollinations were made per cross. In total, nine crosses and 45 pollinations were made per species combination

^z Species combinations in bold were verified as compatible by hybrid seed germination

CROSS				CROSS				CROSS						
fruit set	mericarps	mass	diameter	fruit set	mericarps	mass	diameter	fruit set	mericarps	mass	diameter			
<i>N. humifusa</i> x <i>N. laxa</i>	0.84	0.97	0.72	0.87	<i>N. laxa</i> x <i>N. rupicola</i>	0.15	0.76	0.32	0.82	<i>N. ivaniana</i> x <i>N. plicata</i>	0.84	0.70	0.78	0.94
<i>N. humifusa</i> x <i>N. plicata</i>	0.87	0.94	0.69	0.40	<i>N. plicata</i> x <i>N. humifusa</i>		1.00	0.41	0.69	<i>N. ivaniana</i> x <i>N. aticoana</i>	0.36	0.74	0.51	0.86
<i>N. humifusa</i> x <i>N. aticoana</i>	0.96	0.99	0.63	0.55	<i>N. plicata</i> x <i>N. laxa</i>	0.90	1.00	0.30	0.53	<i>N. ivaniana</i> x <i>N. elegans</i>	0.18	0.76	0.66	0.98
<i>N. humifusa</i> x <i>N. adansonii</i>	0.31	0.98	0.57	0.83	<i>N. plicata</i> x <i>N. aticoana</i>	0.87	0.96	0.88	0.76	<i>N. ivaniana</i> x <i>N. rupicola</i>	0.84	0.76	0.66	0.91
<i>N. humifusa</i> x <i>N. ivaniana</i>	0.78	0.98	0.57	0.84	<i>N. aticoana</i> x <i>N. humifusa</i>		1.00	0.64	0.83	<i>N. elegans</i> x <i>N. laxa</i>	0.55	0.74	0.49	0.67
<i>N. humifusa</i> x <i>N. elegans</i>		1.00	0.49	0.51	<i>N. aticoana</i> x <i>N. laxa</i>	0.41	1.00	0.88	0.91	<i>N. elegans</i> x <i>N. plicata</i>	0.39	0.36	0.37	0.64
<i>N. humifusa</i> x <i>N. rupicola</i>	0.31	0.97	0.51	0.80	<i>N. aticoana</i> x <i>N. plicata</i>	0.85	0.96	0.77	0.50	<i>N. elegans</i> x <i>N. aticoana</i>	0.52	0.26	0.43	0.61
<i>N. laxa</i> x <i>N. humifusa</i>	0.37	0.82	0.80	0.91	<i>N. aticoana</i> x <i>N. adansonii</i>	0.25	0.98	0.80	0.76	<i>N. elegans</i> x <i>N. ivaniana</i>		1.00		0.58
<i>N. laxa</i> x <i>N. plicata</i>	0.79	0.70	0.77	0.92	<i>N. adansonii</i> x <i>N. plicata</i>	0.45	0.83	nd	0.57	<i>N. elegans</i> x <i>N. rupicola</i>	1.00	0.86	0.73	0.77
<i>N. laxa</i> x <i>N. aticoana</i>	0.24	0.87	0.75	0.87	<i>N. adansonii</i> x <i>N. aticoana</i>	0.24	0.81	nd	0.63	<i>N. rupicola</i> x <i>N. elegans</i>	0.78	0.45	0.71	0.84
<i>N. laxa</i> x <i>N. ivaniana</i>	0.84	0.81	0.89	0.97	<i>N. ivaniana</i> x <i>N. laxa</i>	1.00	0.67	0.76	0.94					

Fig. 2 Crossability indices for interspecific hybridizations between *Nolana* species. *Indices* represent performance of interspecific hybridizations compared to intraspecific hybridizations in terms of fruit set, mericarps per fruit, mericarp mass, and

mericarp diameter. An index of 1 indicates that the cross performed as well as intraspecific hybridization. Only those crosses with successful fruit set are represented

any one factor alone. Six species crosses (*N. humifusa* × *N. laxa*, *N. laxa* × *N. plicata*, *N. laxa* × *N. ivaniana*, *N. plicata* × *N. rupicola*, *N. ivaniana* × *N. plicata*, and *N. elegans* × *N. rupicola*) performed nearly as well as intraspecific crosses in all measured factors with all four indices above 0.70. Based on our hypothesis that fruiting and mericarp characteristics are representative of seed set, it is predicted that seed set in these six interspecific crosses is nearly as high as that of intraspecific crosses using the same seed parents. In contrast, two species crosses (*N. elegans* × *N. aticoana* and *N. elegans* × *N. ivaniana*) performed poorly in all measured factors with all four indices falling below 0.50. We hypothesize that these values represent a severe reduction in seed set as compared to intraspecific hybridizations.

Although values analyzed here provide evidence of reduced seed set, they do not confirm such a reduction. To confirm that these external measurements of fruiting success and mericarp characteristics can be used as a reliable measure of reduced fertility of a cross, we had to confirm that mericarp

size is correlated to the number of seeds contained within.

Seed counts by X-ray analysis

Nolana seeds are enclosed within a sclerified mericarp and are not easily removed. A mericarp may be crushed and broken apart to expose enclosed seeds, however this method is highly destructive resulting in nearly 100 % seed death. In order to count seeds within intact mericarps, we used non-destructive X-ray analysis to view mericarp contents. This method worked for all species except *N. laxa* and the crosses where it was used as seed parent, because it was not possible to observe differences in mericarp contrast with the X-ray images.

The number of individual mericarps analyzed per species or per species cross ranged from 12 to 286. Based on differences in contrast within the X-ray images, estimates were made of the number of fully formed seeds and abnormal seeds or empty seed cavities within mericarps. The designation of *full seed*

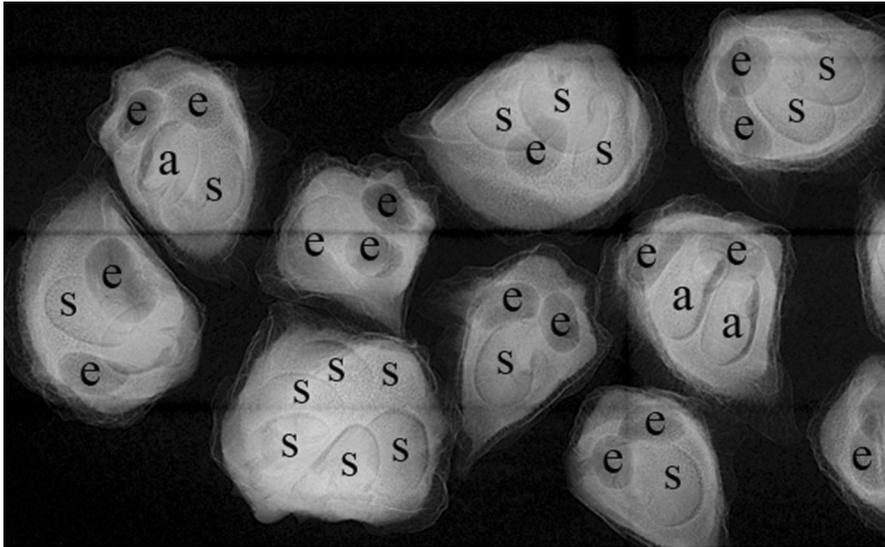


Fig. 3 X-ray image of mericarps resulting from interspecific hybridization of *N. aticoana* × *N. adansonii*. Estimations of seed counts are made based on areas of differing contrast in the image. Cavities are labeled with an ‘s’ to designate a full seed,

‘e’ to designate an empty cavity, and ‘a’ to designate a seed which does not fill its cavity and therefore appears to be abnormally formed

was made when image contrast within a mericarp cavity was bright, indicating the presence of dense tissue. The designation of *empty cavity* was made when contrast of a mericarp cavity was dark indicating vacant space, and that of *abnormal seed* was given when a mericarp cavity appeared to contain some dense tissue but it did not fill the cavity (Fig. 3). Several mericarps were dissected to verify accuracy of designations based on contrast.

Results of X-ray analysis of mericarps are summarized (Table 4). Based on comparison of mericarp size and seed set ratios, we determined that mericarp size was not an effective indicator of seed set. Measurements of mericarp mass and diameter were not related to numbers of full seeds contained within mericarps or to total number of mericarp cavities. Additionally, comparison of seed counts from interspecific hybridizations to those of intraspecific hybridization showed that, on average, fewer full seeds were present in interspecific mericarps, with the exception of crosses involving *N. rupicola* and *N. aticoana* as seed parents. Differences were evident in number of full seeds per mericarp between species crosses. For interspecific crosses, the average number of full seeds per mericarp ranged from zero in *N. plicata* × *N. laxa* to 4 in *N. ivaniana* × *N. rupicola*. Overall, mericarps

of interspecific crosses contained an average of 1.8 full seeds per mericarp. Most interspecific hybridizations resulted in mericarps containing a mix of full seeds and empty cavities or abnormal seeds. Only one cross (*N. ivaniana* × *N. aticoana*) resulted in mericarps with 100 % cavities containing full seeds. On the other hand, ninety-three percent of mericarps from the cross *N. elegans* × *N. laxa* did not contain any full seeds. *N. elegans* × *N. ivaniana*, *N. adansonii* × *N. plicata*, and *N. adansonii* × *N. aticoana* also resulted in high percentages of mericarps without seeds (73, 78, and 86 %, respectively). The first two are species-pairs that are found in different clades, while *N. adansonii* and *N. aticoana* are in the same clade.

Seed germination

Germination of hybrid seed is not a reliable measure of sexual compatibility in *Nolana* due to dormancy barriers. We attempted to germinate all interspecific hybrid seed obtained through this study. Overall, 12 % germination was achieved in terms of seedlings per mericarp, and hybrid seedlings were obtained from 22 of the 32 species crosses for which fruit was produced (Table 5). Germination percentages were generally much lower than the predicted number of seeds within

Table 4 Seed counts in *Nolana* mericarps developed through interspecific hybridization and estimated by X-ray analysis

	Number of mericarps analyzed	Average full seeds per mericarp ^z	Average seed cavities per mericarp ^y	Cavities with full seeds (%)	Mericarps with one or more full seeds (%)
<i>N. humifusa</i>	224	2.5 ± 1.4	4.3 ± 0.9	59	93
<i>N. humifusa</i> × <i>N. laxa</i>	180	2.6 ± 1.5	3.7 ± 1.0	70	89
<i>N. humifusa</i> × <i>N. plicata</i>	120	2.6 ± 1.7	4.0 ± 1.6	65	90
<i>N. humifusa</i> × <i>N. aticoana</i>	109	2.2 ± 1.6	3.7 ± 1.0	59	83
<i>N. humifusa</i> × <i>N. adansonii</i>	63	1.0 ± 0.9	2.9 ± 1.0	33	67
<i>N. humifusa</i> × <i>N. ivaniana</i>	182	2.0 ± 1.5	3.8 ± 1.2	54	83
<i>N. humifusa</i> × <i>N. elegans</i>	14	1.0 ± 1.8	3.1 ± 1.4	33	71
<i>N. humifusa</i> × <i>N. rupicola</i>	60	1.1 ± 1.4	3.3 ± 1.1	34	60
<i>N. plicata</i>	89	5.0 ± 3.0	7.4 ± 3.0	68	97
<i>N. plicata</i> × <i>N. humifusa</i>	24	2.1 ± 1.4	4.5 ± 0.7	46	92
<i>N. plicata</i> × <i>N. laxa</i>	12	0	8.6 ± 3.2	0	0
<i>N. plicata</i> × <i>N. aticoana</i>	115	3.6 ± 2.2	5.0 ± 2.6	72	97
<i>N. aticoana</i>	228	1.9 ± 1.8	3.9 ± 1.2	48	64
<i>N. aticoana</i> × <i>N. humifusa</i>	27	2.2 ± 1.5	3.8 ± 1.3	59	89
<i>N. aticoana</i> × <i>N. laxa</i>	100	2.5 ± 1.2	4.1 ± 1.6	61	96
<i>N. aticoana</i> × <i>N. plicata</i>	84	3.4 ± 1.8	4.3 ± 1.8	80	96
<i>N. aticoana</i> × <i>N. adansonii</i>	38	1.7 ± 1.0	4.1 ± 0.9	41	87
<i>N. adansonii</i>	139	0.9 ± 0.4	1.1 ± 0.2	81	84
<i>N. adansonii</i> × <i>N. plicata</i>	177	0.2 ± 0.5	1.1 ± 0.3	22	22
<i>N. adansonii</i> × <i>N. aticoana</i>	93	0.2 ± 0.5	1.1 ± 0.2	16	14
<i>N. ivaniana</i>	50	4.2 ± 1.8	5.2 ± 1.5	81	94
<i>N. ivaniana</i> × <i>N. laxa</i>	214	3.1 ± 2.1	4.1 ± 2.0	74	92
<i>N. ivaniana</i> × <i>N. plicata</i>	88	2.8 ± 2.2	4.1 ± 1.7	68	76
<i>N. ivaniana</i> × <i>N. aticoana</i>	39	2.2 ± 1.6	2.2 ± 1.9	100	87
<i>N. ivaniana</i> × <i>N. elegans</i>	19	3.7 ± 1.8	4.7 ± 1.9	78	95
<i>N. ivaniana</i> × <i>N. rupicola</i>	78	4.0 ± 1.7	4.6 ± 1.7	88	100
<i>N. elegans</i>	112	2.4 ± 1.8	4.0 ± 2.2	61	94
<i>N. elegans</i> × <i>N. laxa</i>	27	0.1 ± 0.3	1.6 ± 0.7	5	7
<i>N. elegans</i> × <i>N. plicata</i>	35	0.6 ± 0.7	2.3 ± 1.3	28	57
<i>N. elegans</i> × <i>N. aticoana</i>	82	0.5 ± 0.6	2.1 ± 1.1	22	40
<i>N. elegans</i> × <i>N. ivaniana</i>	51	0.3 ± 0.5	1.5 ± 0.7	18	27
<i>N. elegans</i> × <i>N. rupicola</i>	286	1.8 ± 1.5	2.8 ± 2.0	66	86
<i>N. rupicola</i>	82	1.1 ± 1.0	1.6 ± 1.0	69	77
<i>N. rupicola</i> × <i>N. elegans</i>	277	1.5 ± 1.1	1.9 ± 1.2	76	85

^y Seed cavities were identified in the X-ray images based on contrast. This count includes those cavities appearing to contain full seeds, those which appear empty, and those which appear to contain abnormal seeds. Value represents the average count of all analyzed mericarps ± SD

^z Designation of 'full seed' based on contrast in X-ray image. This value represents the number of cavities visible in the X-ray image appearing full. Value represents the average count of all analyzed mericarps ± SD

the sown mericarps as determined by X-ray analysis. In crosses with germination, percentages ranged from 0.5 % in *N. humifusa* × *N. laxa* to 68 % in *N.*

elegans × *N. aticoana*. In many cases, germination percentages were higher for interspecific crosses than for seed obtained from intraspecific hybridizations.

Table 5 Germination of *Nolana* intraspecific and interspecific seed

Species or hybrid family ^z	Seeds per mericarp					
	Range ^x	Mean ^y	Mericarps sown	Estimated seeds sown	Seedlings germinated	Germination % ^y
<i>N. humifusa</i>	1–5	2.7	200	540	17	3
<i>N. humifusa</i> × <i>N. adansonii</i>	0–5	1.0	68	65	4	6
<i>N. humifusa</i> × <i>N. aticoana</i>	0–6	2.2	98	214	18	8
<i>N. humifusa</i> × <i>N. elegans</i>	0–2	1.0	15	15	0	0
<i>N. humifusa</i> × <i>N. ivaniana</i>	0–9	2.0	172	346	3	1
<i>N. humifusa</i> × <i>N. laxa</i>	0–5	2.6	183	472	1	0
<i>N. humifusa</i> × <i>N. plicata</i>	0–8	2.6	93	240	54	23
<i>N. humifusa</i> × <i>N. rupicola</i>	0–9	1.1	69	77	0	0
<i>N. plicata</i>	1–4	5.3	80	424	4	1
<i>N. plicata</i> × <i>N. aticoana</i>	0–11	3.6	117	418	8	2
<i>N. plicata</i> × <i>N. humifusa</i>	0–5	2.1	25	52	0	0
<i>N. plicata</i> × <i>N. laxa</i>	0	0.0	12	0	0	0
<i>N. aticoana</i>	1–7	3.1	140	434	24	6
<i>N. aticoana</i> × <i>N. adansonii</i>	0–4	1.7	54	91	19	21
<i>N. aticoana</i> × <i>N. humifusa</i>	0–6	2.2	25	56	4	7
<i>N. aticoana</i> × <i>N. laxa</i>	0–5	2.5	87	216	0	0
<i>N. aticoana</i> × <i>N. plicata</i>	0–10	3.4	115	396	52	13
<i>N. adansonii</i>	1–2	1.0	100	100	10	10
<i>N. adansonii</i> × <i>N. aticoana</i>	0–2	0.2	124	21	0	0
<i>N. adansonii</i> × <i>N. plicata</i>	0–2	0.2	240	58	0	0
<i>N. ivaniana</i>	1–8	4.5	46	207	5	2
<i>N. ivaniana</i> × <i>N. aticoana</i>	0–5	2.2	39	84	8	10
<i>N. ivaniana</i> × <i>N. elegans</i>	0–6	3.7	20	74	0	0
<i>N. ivaniana</i> × <i>N. laxa</i>	0–8	3.1	173	528	4	1
<i>N. ivaniana</i> × <i>N. plicata</i>	0–7	2.8	94	260	28	11
<i>N. ivaniana</i> × <i>N. rupicola</i>	1–7	4.0	93	372	18	5
<i>N. elegans</i>	1–7	2.7	100	270	6	2
<i>N. elegans</i> × <i>N. aticoana</i>	0–2	0.5	55	50	34	68
<i>N. elegans</i> × <i>N. ivaniana</i>	0–1	0.3	57	15	9	60
<i>N. elegans</i> × <i>N. laxa</i>	0–1	0.1	109	8	1	13
<i>N. elegans</i> × <i>N. plicata</i>	0–3	0.6	45	28	15	54
<i>N. elegans</i> × <i>N. rupicola</i>	0–8	1.8	459	835	106	13
<i>N. rupicola</i>	1–13	1.5	62	93	2	2
<i>N. rupicola</i> × <i>N. elegans</i>	0–7	1.5	261	386	0	0
Total	0–13	1.8	3630	7420	454	12

^y Germination calculated as the number of seedlings germinated divided by the estimated number of seeds sown

^z Seeds per mericarp estimates made by X-ray analysis of a random set of mericarps from each species or hybrid family

Species pairs verified as sexually compatible by germination of hybrid seed have been noted in bold print in Table 3. In most cases, compatibility in those species appearing to be highly compatible as implied

by fruit set values was verified by seed germination. An exception to this is the cross *N. rupicola* × *N. elegans*. Despite high fruiting success and seemingly high seed set, no seeds germinated from this cross. The

reciprocal cross, however, had 13 % germination success.

Many *Nolana* species live in sympatry and have overlapping ranges. Nonetheless, documented natural hybrids are very rare (Tago-Nakazawa and Dillon 1999) with the exception of only one case observed involving *N. pilosa* and *N. thinophila* (R. Freyre, pers. obs.). Postzygotic reproductive isolating barriers are likely maintaining species identity, even in close proximity (Jewell et al. 2012). Interestingly, in this study we have documented sexual compatibility in species pairs that were found growing in close proximity.

Based on seed germination, we have confirmed compatibility in several combinations in which species belong to different clades based on molecular analyses: *N. humifusa* × *N. ivaniana*, *N. humifusa* × *N. plicata*, *N. plicata* × *N. aticoana* (and reciprocal), *N. ivaniana* × *N. aticoana*, *N. ivaniana* × *N. rupicola*, *N. elegans* × *N. laxa*, and *N. elegans* × *N. plicata*. Interestingly, the combination *N. elegans* × *N. aticoana* had 68 % germination success, even though the species are in different clades and also have the most geographic distance.

There were ten species pairs for which fruit set was successful but where germination failed, and a definitive designation of sexual compatibility cannot be assigned. We cannot know whether failure of germination was caused by nonviable seed or seed dormancy. Nevertheless, germination of hybrid seed has provided verification of sexual compatibility between 18 species combinations previously not known to be compatible.

Conclusions

Our studies of sexual compatibility within and between *Nolana* species have expanded the current knowledge regarding sexual compatibility within the genus. We found species to be generally self-incompatible, with limited self-compatibility seen in two species (*N. adansonii* and *N. ivaniana*). Intraspecific compatibility is strong within species with few exceptions. Interspecific compatibility is common. Fruit set was achieved in 32 out of 56 species crosses, and hybrid seedlings were obtained from 22 of these crosses. Fruit set success ranged from 7 to 96 %, with an average of 44 % fruiting success out of all attempted pollinations.

Using fruit set as an initial indicator of possible compatibility between species, we classified 24 species crosses as incompatible based on unsuccessful fruit production. Twelve of these species crosses were bilaterally incompatible and 12 were unilateral with reciprocals exhibiting various degrees of fruiting set success from 7 to 78 %. We observed reduced compatibility in interspecific hybridizations as reduced fruiting success and reduced seed counts as compared to values obtained through intraspecific hybridizations.

X-ray analysis showed that mericarp size is not proportional to number of seeds contained within. Large mericarps often contained a small number of seeds or no seeds. Therefore, measurement of mericarp mass and diameter cannot be used to estimate relative levels of seed set success. X-ray technology, however, proved to be a valuable tool in analysis of sexual compatibility within *Nolana*. Overall, 30 % of the analyzed mericarps were found to contain no seeds. These findings illustrate the importance of seed set evaluations in analyses of sexual compatibility in *Nolana*.

Previous to these studies, sexual compatibility was known to exist only between *N. paradoxa* and *N. humifusa*, *N. elegans*, *N. rupicola*, and *N. aplocaryoides*. Eighteen new species combinations have been verified compatible. The compatible combinations include species that have been placed in different clades based on molecular analyses. Results of these studies are a significant contribution to the current knowledge of sexual compatibility within *Nolana* and are important for future breeding efforts.

Acknowledgments This research was funded by a NHIRC Cooperative Agreement between the University of New Hampshire and Pleasant View Gardens, Loudon, NH. The authors would like to thank Dr. Paul Fisher and Dr. Tom Davis for reading earlier versions of this article; Dr. Michael Dillon for providing us with some of the plant material; Dr. David Tay for help with X-ray analysis; Dr. Kent Bradford and Dr. Rob Griesbach for their advice and encouragement; John McLean, Evan Ford, David Goudreault, and Russell Norton for plant maintenance; and Deb Schneider, Steve Henniger and Ryan Lockheart for help in data collection and propagation.

References

- Bondeson WE (1986) Gynoecial morphology and funicular germination plugs in the Nolanaceae. *Nord J Bot* 6:183–198

- Campin MG (1925) A cytological study of pollen development in *Nolana*. *New Phytol* 24:17–23
- Datta S (1933) Embryological and cytological studies in *Nolana atriplicifolia* and *N. prostrata*. *J Ind Bot Soc* 12:131–152
- de Nattencourt D (1977) Incompatibility and incongruity in wild and cultivated plants. Springer, New York
- di Fulvio TE (1969) Embriología de *Nolana paradoxa* (Nolanaceae). *Kurtziana* 5:39–54
- di Fulvio TE (1984) Número cromosómico de *Nolana rostrata*. *Kurtziana* 17:169
- Dillon MO, Nakazawa M, Leiva S (2003) The lomas formations of coastal Peru: composition and biogeographic history. In: Haas J, Dillon MO (eds) *El Niño in Peru: biology and culture over 10,000 years*. *Fieldiana: Botany, N.S.* 43, pub. 1524, p 1–9
- Dillon MO (2005) Solanaceae of the lomas formations of coastal Peru and Chile, p. 131–155. In: Keating RC, Hollowell VC, Croat TB (eds) *A festschrift for William G. D'Arcy: the legacy of a taxonomist*. Missouri Botanical Garden Press, St. Louis
- Dillon MO, Tu T, Xie L, Quipuscoa Silvestre V, Wen J (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian deserts along the western coast of South America. *J Syst Evol* 47:457–476
- Douglas AC, Freyre R (2010) Floral development, stigma receptivity and pollen viability in eight *Nolana* (Solanaceae) species. *Euphytica* 174:105–117
- Ehlenfeldt MK, Hanneman RE (1988) Genetic control of endosperm balance number (EBN): three additive loci in a threshold-like system. *Theor Appl Genet* 75:825–832
- Freyre R, Douglas AC, Dillon MO (2005) Artificial hybridizations in five species of Chilean *Nolana* (Solanaceae). *HortScience* 40:532–536
- Jewell C, Douglas Papineau A, Freyre R, Moyle LC (2012) Patterns of reproductive isolation in *Nolana* (Chilean Bellflower). *Evolution* 66:2628–2636
- Knapp S (2002) Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. *J Expt Bot* 53:2001–2022
- Olmstead RG, Palmer JD (1992) A chloroplast DNA phylogeny of the Solanaceae: subfamilial relationships and character evolution. *Ann Mo Bot Gard* 79:346–360
- Ortiz R, Ehlenfeldt MK (1992) The importance of endosperm balance number in potato breeding and the evolution of tuber-bearing *Solanum* species. *Euphytica* 60:105–113
- Pozzobon MT, Schifino-Wittman MT, Bianchetti L (2006) Chromosome numbers in wild and semidomesticated Brazilian *Capsicum* L. (Solanaceae) species: do $x = 12$ and $x = 13$ represent two evolutionary lines? *Bot J Linn Soc* 151:259–269
- Saunders ER (1934) The history, origin and characters of certain interspecific hybrids in *Nolana* and their relation to *Nolana paradoxa*. *J Genet* 29:387–419
- Tago-Nakawaza M, Dillon MO (1999) Biogeografía y evolución en el clado *Nolana* (Solaneae-Solanaceae). *Arnaldia* 6:81–116
- Tu T, Dillon MO, Sun H, Wen J (2008) Phylogeny of *Nolana* (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four plastic markers and the nuclear LEAFY second intron. *Mol Phylogenet Evol* 49(2): 561–573