



Sex morphs and invasiveness of a fleshy-fruited tree in natural grasslands from Argentina

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Sex morphs and invasiveness of a fleshy-fruited tree in natural grasslands from Argentina

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Abstract

Invasiveness has usually been studied as a species-level attribute; nevertheless, phenotypic differences between individuals in a population can lead to significant variations in colonization ability. In this paper, we analyse the potential effects of sex morphs of *Prunus mahaleb*, a gynodioecious fleshy-fruited tree, on its invasiveness in natural grasslands in the southern Argentine Pampas. We assessed the abundance of both hermaphrodite and female plants and compared their fecundity, propagule size and germination response. We found that the females were less abundant in the invasive populations studied, apparently since the beginning of the colonization. However, our results demonstrated that at the present time females do not show any fecundity reduction, which clearly shows that *P. mahaleb* has established an effective interaction with generalist pollinators which compensates for the apparent disadvantage of females. Fruit set showed a wider range of variability over time in the females than in the hermaphrodites, which could be the consequence of greater susceptibility to changes in the activity of pollinators. We found no evidence of a female benefit due to reallocation of resources or better outcrossed progeny considering propagule size and germination. We discuss the relative importance of sex morphs and interactions at different stages of the invasion process.

Keywords: invasiveness, *Prunus mahaleb*, gynodioecy, fecundity, grasslands

Introduction

Invasive species, among other novel organisms, constitute a central component of global change and are considered a major threat to biodiversity, human health and food security (Jeschke et al. 2013; Simberloff et al. 2013). Invasiveness, the capacity to establish and spread in new habitats, has been classically assessed at the species level (Rejmánek and Richardson 1996). However, some authors have recently emphasized the need to include the effects of genetic characteristics of provenances and populations in the analysis of invasiveness (McCormick et al. 2010; Zenni et al. 2014). It has been demonstrated that individual plants can contribute differentially to future generations and biological invasions can be promoted by high-performance genotypes in the introduced pool that produce a disproportionate fraction of the offspring (Matesanz and Sultan 2013; Zenni et al. 2014). The sex morph could be particularly critical in modulating the ability of an individual to successfully produce and disperse its propagules, and sex ratio may affect the rate of spread of the invasive population.

Gynodioecy is a widespread sexual system characterizing species that contain two genetically determined morphs, hermaphrodites and female individuals (Bailey and Delph 2007; Dufay and Billard 2012). The genetic control of this sex dimorphism is often the result of a complex interaction between maternally inherited male sterility genes in the mitochondrial genome and the bi-parentally inherited male fertility restorer nuclear genes (Dufay and Pannell 2010). In species with this breeding system, the female individuals have obvious disadvantages compared to the hermaphrodites, because they only achieve fitness via female function and they are dependent on the arrival of external pollen for seed set (Bailey and Delph 2007). Conversely, females can benefit from the enhanced fecundity due to reallocation of resources from pollen to seed

production and can produce better outcrossed progeny that do not show the effects of inbreeding depression (Horovitz and Beiles 1980; Dufay and Pannell 2010; Dufay and Billard 2012).

Compared to hermaphrodites, female plants can be negatively affected in pollen-limited scenarios, as is expected in colonizing populations. “Baker’s law” states that self-fertilization should be advantageous in colonizing scenarios, where pollinators or partners for mating may be scarce (Baker 1965; Cheptou 2011; Chaney and Baucom 2012). Female plants are expected to have a lower fecundity because of their dependence on pollinators. In the other hand, hermaphrodites will be able to reproduce more during the early stages of invasion if they can self-fertilise, which could result in greater chances of colonization (Hao et al. 2011; Cheptou 2011; Moodley et al. 2015). In this sense, facilitation interactions and Allee effects have been highlighted as important factors affecting invasion processes (McCormick et al. 2010). In gynodioecious species, Allee effects might encompass a range of mechanisms associated with pollen limitation that can affect their ability to colonize and spread.

Theoretical modelling has shown that the sex ratio in gynodioecious populations can vary depending on the balance between the compensation effects that benefit the females and the costs of negative pleiotropic effects of restoration in hermaphrodites (Bailey et al. 2003; Bailey and Delph 2007). Female individuals are expected to represent up to 50 % of populations, but this proportion depends on the effects already mentioned (Shykoff et al. 2003; Dufay and Billard 2012). In invasive populations, the sex ratio at the colonization stage, explained on the basis of the founder effect, could affect the rate of spread, and even the fate of the invasion. An initial predominance of female individuals could slow population growth and dispersal, even increasing the chances of invasive failure due to random factors. Once the population exceeds a

minimum size, a high proportion of females could help to maintain high levels of genetic variability and a high production of propagules, provided efficient pollination interactions are established with the local fauna.

Natural grasslands in the South American Pampas have suffered severe alterations and only a small proportion of the original surface is protected in nature reserves (Bilenca and Miñarro 2004). The proliferation of invasive trees and shrubs represents a major threat for biodiversity and ecosystem functioning as in other grasslands and savannas worldwide (Richardson et al. 2014). A set of invasive woody plants of diverse origins have spread in the last remnants of natural grasslands in the Pampas eco-region, among them St. Lucie's cherry (*Prunus mahaleb*) has established dispersal interactions with the local fauna (Zalba and Villamil 2002; Amodeo and Zalba 2013).

In this paper, we assess the relative abundance of the two sex morphs in the *Prunus mahaleb* populations invading natural grasslands in the southern Argentine Pampas, we analyze differences in fecundity, propagule size and germination response between them and discuss the effects of the sex ratio on the spread of the invasion. Fruit set in female plants depends entirely on pollinators, so we expect them to show a lower fecundity, especially in a colonization scenario like this. On the other hand, if hermaphrodites are negatively affected by self-fertilization or females benefit from reallocation of resources or better outcrossed progeny, the latter are expected to show larger propagules and enhanced germination rates.

Materials and methods

Study species

St. Lucie's cherry (*Prunus mahaleb* L.) is a small fleshy-fruited tree native to Eurasia, frequently used as a rootstock for orchard trees, as an ornamental plant and also for wood production (Bass et al. 2006; Grisez et al. 2008). These have been the reasons that drove its introduction in several countries, resulting in invasion processes in some of them, including the United States (Swearingen 2008), Canada (Brouillet et al. 2010), Australia (Bass et al. 2006), New Zealand (Webb et al. 1988) and Argentina (Zalba and Villamil 2002). The presence of this species in the Pampas has been reported for more than 50 years (Zalba 2001) and we have estimated the age of the oldest tree in the study area to be 62 years (Zalba and Amodeo 2015). The species has spread over natural grasslands, threatening remnants of high value for biodiversity conservation (Zalba and Villamil 2002; Amodeo and Zalba 2013; Zalba and Amodeo 2015).

The phenology of this species in the study area is similar to that reported for its native range (Guitián et al. 1992; Jordano 1993) and is characterized by short flowering periods (19-22 days) with a high synchronization between individuals and populations (Amodeo and Zalba 2015). Corymbs of 3-10 small white flowers, with numerous stamens, are produced by mid spring and are visited by bees, flies, butterflies and beetles. The species is partially self-compatible and Jordano (1993) reported preliminary evidence in favour of a situation of stable gynodioecy in wild *P. mahaleb* populations, resulting from the interaction of cytoplasmic male sterility genes and nuclear restorers. Production and ripening of fleshy fruits take place during approximately two months in late spring and early summer (Marañón et al. 2004;

Amodeo and Zalba 2015) and seed dispersal depends strongly on birds and mammals (Jordano and Schupp 2000; Amodeo and Zalba 2013).

Study site

This study was carried out at the Ernesto Tornquist Provincial Park, which covers an area of approximately 6,700 ha of mountain grasslands in the southern Argentine Pampas (38° 3.90'S; 61° 58.33'W). This reserve is one of the few protected areas in the whole eco-region that includes remnant grasslands in good conservation status (Zalba and Villamil 2002; Bilenca and Miñarro 2004). The climate is temperate, with a mean annual temperature of 14° C and broad daily and annual fluctuations. The annual precipitation is around 800 mm, mostly concentrated in spring (Burgos 1968). The park includes 37 endemic species and a high proportion of the plant diversity present in the Pampas eco-region (Long and Grassini 1997; Bilenca and Miñarro 2004). The vegetation is dominated by steppe alternating with rocky outcrops. Exotic woody plants, including pines, brooms, acacias and eucalypts, can be found growing in small plantations, spontaneous clumps or as isolated individuals (Zalba and Villamil 2002). This study was carried out in four populations. Two of them were located in a shaded habitat, in the understory of stands dominated by other exotic trees, such as *Eucalyptus camaldulensis* Dehnh., *Pinus canariensis* C. Sm., *Ulmus pumila* L. and *Populus alba* L. (20-25 m height, densities between 32 and 450 ind./ha). The understory was mainly composed of *Conium* sp., *Hedera helix* L., *Carduus* sp., *Bromus* sp., *Prunus mahaleb* and *Genista monspessulana* (L.) L.A.S. Johnson. The other two populations were located in open habitats dominated by grasses and herbs under 50 cm height, including *Stipa* sp., *Aristida* sp., *Briza* sp., *Eryngium* sp., *Abutilon terminale* (Cav.) A.St.-Hil., *Discaria americana* Gillies & Hook and *Helenium* sp.

Abundance, fecundity and fruit set

During September 2010, a total of 302 trees were studied in four populations (two in shaded habitat and two in open habitat), including reproductive and non-reproductive individuals. In each population, the sex morph was assessed by careful inspection of the flowers of every reproductive individual within a sampling transect of 500-800 m in length and four meters wide. The number of individuals of each sex morph was recorded for each population and its proportion was calculated and compared with values reported in the native range of the species (Jordano 1993).

In order to study the reproductive performance, we marked 52 trees from the populations located in open habitats (the most common situation for *P. mahaleb* in the study area) and they were monitored during three consecutive fruiting seasons (2010-2011, 2011-2012 and 2012-2013). The trees were selected so that the full range of tree sizes was included for each sex morph. From previous studies, we know that the basal stem diameter is a good predictor of the tree age (Zalba and Amodeo 2015). When the fruits began to ripen during early December, the basal stem diameter (*BSD*, in cm) was measured and the total number of ripe and unripe fruits produced per plant was determined over three years. For small plants (basal stem diameter < 10 cm), a complete count of the fruit on all branches was recorded. For larger trees, the fruit in a sample of two to six marked branches distributed in all quadrants was counted. The number of branches selected was defined seeking an effort directly proportional to the crown volume, using the formula: $N_B = 2 * \log C_V$; $N_B = \text{Number of branches}$, $C_V = \text{Crown volume}$. The proportional representation of the total crown volume was recorded for each branch and the count value of each one was extrapolated to give the total number of fruits in the tree. The mean for all the branches sampled for each tree was calculated. During September 2010 and 2012, the number of flowers was also

counted and the fruit set (the proportion of final fruits produced in relation to the initial number of flowers) was calculated.

Propagule size and germination

During the fruiting season 2010-2011, 280 fruits were collected from the branches of a subsample of the marked trees (24 trees of different sizes and sex morphs). Ten fruits and their stones of each tree were measured using a Vernier digital calliper (0.01 mm resolution) and weighed using a digital balance (0.0001 g resolution), maximum length (L), maximum diameter (D_1), perpendicular diameter (D_2) and wet mass. The fruit and stone volumes (V) were calculated considering an ellipsoidal shape: $V = \frac{\pi * L * D_1 * D_2}{6}$.

A germination test was carried out for 18 trees (11 hermaphrodites and seven females) to analyze the effect of plant size and sex morph. Five groups were defined based on the basal stem diameter and sex morph, mixing the fruits of 3-4 trees in each category. The fruits were stored in paper bags for 90-100 days after collection, and then the stones were immersed in water during 24-48 hours and kept in wet sand for 96-99 days of cold stratification (4-6°C, 100% RH, Grisez et al. 2008; Pipinis et al. 2012). Although scarification has been reported to enhance *P. mahaleb* germination, no scarification was performed in order to ensure equal treatment to all the seeds. The stones were treated with zinc ethylenebisdithiocarbamate fungicide powder (CENET 2008) and then placed in a germination chamber (14 hs light 20°C / 10 hs dark 10°C, 50-70% RH) in Petri dishes with a sand substrate in 4-6 replicates of 20 seeds each per group (procedure based on preliminary tests and Grisez et al. 2008; Prada and Arizpe 2008). Seeds were checked every two days for a total period of 45 days and germination was considered to occur when the radicle had emerged 2 mm. After this, viability tests

were carried out on those seeds that did not germinate using a solution of triphenyltetrazolium chloride 0.03% for 18-24 hours in subdued light conditions at 20°C.

During 2012, a germination test in an experimental garden was carried out at the Pillahuinco Botanic Garden (Ernesto Tornquist Provincial Park) using stones collected from 12 hermaphrodites and six female trees of different sizes. After 80-90 days of storage in paper bags, two replicates of ten seeds per tree were sown individually at a depth of 1 cm in cylindrical cardboard pots (5 x 10 cm). Pots were protected from predators using a wire mesh and they were exposed to sunlight and rainfall. In early spring, the number of seedlings in each group was recorded.

Statistical analysis

The variations of fecundity (mean number of fruits and flowers produced by a tree) and fruit set with respect to basal stem diameter (*BSD*, log transformed) and sex morph were analysed using Generalized Linear Models. A negative binomial distribution with *log* link function was used for the total number of fruits and flowers produced and a binomial distribution with *logit* link function was used for fruit set. The models included *BSD*, sex morph and their interaction, as well as the population, for evaluating the potential differences between groups. Only plants with *BSD* of less than 25 cm were included in the analyses, to ensure similar size range for both sex morphs (as females with *BSD* greater than 25 cm were absent from the dataset). The relevance of these variables was analysed using a Likelihood Ratio Test comparing sequentially alternative models (Bolker 2008). The variation in volume and weight of the fruits and stones in relation to *BSD* and sex morph was analysed using Generalized Linear Models with a Gaussian distribution and *log* link function. Germination and viability of seeds were analyzed by means of Generalized Linear Models with binomial distribution and

logit link function (Peng et al. 2002), including *BSD*, sex morph and the interaction term. We checked the assumptions for all the models by analyzing plots of residuals and influential observations. All analysis and graphs were performed using R and packages *stats* (R-Core-Team 2014) and *ggplot2* (Wickham 2009).

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Results

*Abundance, fecundity and fruit set*¹

P. mahaleb was the dominant shrub species in all populations, co-existing with small stands of *Spartium junceum* L., *Genista monspessulana*, and isolated individuals of *Rubus ulmifolius* Schott and *Rosa rubiginosa* L. The total density of *P. mahaleb* ranged from 154.7 to 281.5 individuals/ha, with a dominance of non-reproductive individuals (under four-year-old individuals in Table 1) and similar age structures in the populations studied. Female trees represented 5.23% of the total reproductive individuals in the populations (range= [1.85% - 11.63%], n=4, Table 1), a figure significantly different from the 1:1 ratio reported for its native range ($\chi^2 > 30.08$, $p < 0.01$, Jordano 1993). Hermaphrodites showed bright orange-yellow globose anthers filled with pollen, while female plants presented shrunken pink anthers with no pollen (Figure 1).

Table 1 near here

Figure 1 near here

Basal stem diameter varied between 3 cm and 21 cm for female plants, and between 2.5 cm and 50 cm for hermaphrodites. In spite of our efforts, no larger female plants were found. The population factor was not significant hence it was discarded from all the models (Likelihood Ratio Tests, $p > 0.8$). The total number of flowers produced by a plant ranged from 46 to 1,134,432 and it showed a positive relationship with the basal stem diameter of the tree. The total number of fruits produced ranged from six to 299,977, also showing a positive relationship with the basal stem

¹ Data set available in supplementary Table S1

diameter. We found no effect of sex morph on the flower or fruit production (Table 2). In the case of fruit production, the interaction of gender and basal stem diameter was only significant in 2012. Due to the rare and weak evidence for this interaction, we show the simplest model describing the variation of fruit production across the full range of basal stem diameters for all the trees studied (Figure 2).

Table 2 near here

Figure 2 near here

Only about one in 3.5-4 flowers produced fruit, with an average fruit set of 0.251 (range= [0.197-0.575], n= 51) for 2010 and 0.288 (range= [0.035-0.517], n= 17) for 2012. Fruit set varied significantly with respect to the basal stem diameter and sex morph, showing an interaction between both variables for the two seasons studied (Table 2). For hermaphrodites, the fruit set decreased in relation to the basal stem diameter showing similar results in the two seasons (Figure 3). For 5-cm-BSD hermaphrodite plants, the predicted fruit set was 0.281 (SE=0.0011) and 0.254 (SE=0.0013) for 2010 and 2012, respectively. For 20-cm-BSD plants, these values were reduced to 0.199 (SE=0.0004) and 0.216 (SE=0.0008), respectively. On the contrary, the pattern of this relationship in the females differed between seasons. For 5-cm-BSD female plants, the predicted fruit set was 0.247 (SE=0.0014) and 0.334 (SE=0.0039) for 2010 and 2012, respectively. For 20-cm-BSD plants, this value was reduced to 0.225 (SE=0.0005) for 2010 and it increased to 0.411 (SE=0.001) in 2012.

Figure 3 near here

Propagule size and germination

The volume and weight of the fruits did not differ significantly in relation to the sex morph but it increased significantly in relation to the basal stem diameter. The volume and weight of stones did not vary significantly in relation to the sex morph or basal stem diameter (Table 3).

Fewer than half the tested seeds germinated under laboratory conditions, with an overall mean germination rate of 0.285 (SE= 0.055). Germination varied with tree size as a main effect and in interaction with the sex morph (Table 3). Seeds from small plants germinated better than those from large trees, and seeds from small female trees tended to germinate better than those from small hermaphrodite ones, whereas those from larger hermaphrodites tended to germinate better than those from the larger females. Seed viability decreased with tree size (Figure 4). The germination rate in experimental garden varied between 0.55 and 0.9 for all plants (mean value= 0.728, SE=0.021) and did not show any significant differences with respect to the basal stem diameter or sex morph of the mother plant (Table 3).

Table 3 near here

Figure 4 near here

Discussion

Our results reflect the relative importance of sex morphs and the interaction with generalist pollinators at different stages of the invasion process for gynodioecious plants. We found that females were less represented than hermaphrodites in the invasive populations studied, apparently since the beginning of the colonization. The initial preponderance of hermaphrodites could have fostered the early stages of invasion, when low tree densities probably limited the pollen flow for females, if any were present. Nevertheless, our results also demonstrate that at the present time females do not show any sign of fecundity reduction, which clearly shows that *P. mahaleb* has established an effective interaction with generalist pollinators which compensates for the apparent female disadvantage. The latter is consistent with a greater inter-annual variability in fruit set by females that could respond to changes in pollinator abundance or activity. On the other hand, we found no evidence of a female benefit due to reallocation of resources or any better outcrossed progeny considering propagule size and germination.

Regarding to the sex-morph ratio, the female type represents an average of 5% total plants in the populations at the present time, not exceeding 12% in any case. The proportion of female plants reported in native populations in southern Spain was 42.16% (n=43) and 44.74% (n=76, Jordano 1993). These proportions did not differ from a 1:1 ratio, a significantly higher value in comparison with the ratio we found in our invasive populations. This difference could just be a consequence of a founder effect, originating from a higher proportion of hermaphrodites in the introduced stock. This is consistent with the absence of female individuals among the largest trees, which can be considered the founding individuals in the study area according to previous studies (Zalba and Amodeo 2015). It has been highlighted that the morphs that are

capable of self-fertilization have a key effect on the successful establishment and invasion (Rambuda and Johnson 2004; Hao et al. 2011). The initial preponderance of hermaphrodites could have been crucial for the fate of *P. mahaleb* invading grasslands in the Pampas.

Female plants did not show any signs of fecundity reduction during this study. The effect of sex morph and its interaction with basal stem diameter was not important in the models, indicating that the fecundity of hermaphrodites and female plants did not differ significantly when the plant size was considered. This indicates the establishment of effective pollination interactions with either native or global pollinators. As has been highlighted for other invasive plants, the acquisition of effective mutualistic interactions is a key component promoting the invasion process (Richardson and Pyšek 2006; Saul and Jeschke 2015). There are some *P. mahaleb* traits that might be key in determining its ability to rapidly establish these novel interactions, like the short duration and high synchronization of flowering that increases the attraction of multiple generalist pollinators and hence cross-pollination (Howe 1993; Jordano 2000; Amodeo and Zalba 2015).

The females showed greater variation in fruit set than the hermaphrodites, but this difference did not affect the overall fecundity. The average fruit set values that we recorded (0.25-0.28) are in accordance with those reported in the species native range of distribution (0.1-0.3, Guitian 1993; 1994; Jordano 1993). Similar values have been reported for other members of the genus (Sutherland and Delph 1984). Selective fruit abortion and ovary reserve have been proposed as the most suitable explanations for these low values in this species (Guitian 1993; 1994). The variation of fruit set in response to tree size and season was wider for females (ranging from 0.22 to 0.41) than for hermaphrodites (0.19 to 0.28) in our study. Spatial or temporal shifts in the

pollinator community may alter the difference in the pollination success between sex morphs (Alonso and Herrera 2008). Jordano (1993) reported a significant reduction in fruit set of *P. mahaleb* females during years with persistent adverse weather during pollination in southern Spain. This hypothesis of pollinator limitation for fruit set in females was also supported by experimental pollination tests (Jordano 1993). The author found that open-pollinated flowers of hermaphrodites resulted in a greater mean fruit set (0.291) than in females (0.253). The addition of cross pollination significantly enhanced fruit set over controls only in the female trees, and the reductions in fruit set in the hermaphrodites during unfavourable years were less severe than in the females. The wider variation observed in our invasive populations might reflect a stronger dependency on the activity of pollinators and environmental conditions in females.

Our results do not show any evidence of a female benefit due to reallocation of resources or better outcrossed progeny. We did not find any significant differences in the size and weight of fruits and seeds between sex morphs. Germination tests in the laboratory and under natural conditions did not show any differences in viability and germination capacity either. Similar results have been reported for other gynodioecious shrubs (Alonso and Herrera 2001) and for native populations of *P. mahaleb* (Jordano 1993). Our laboratory tests showed that the size of the mother plant has an effect on germination and viability, reaching very low values for large plants with a basal stem diameter above 35 cm. Although there is a significant interaction between plant size and sex morphs, the main differences seem to be related to the former. Under natural conditions, however, this effect was not noticeable. Large plants might produce seeds with a harder endocarp than younger plants because of allometric changes in the endocarp development, resulting in greater coat dormancy (Fenner 1991; Gutterman 2000). This effect might have been relevant in the laboratory where the stones were not

scarified. On the other hand, this effect of a harder endocarp might have been neutralized by the action of soil microorganisms and weathering in our experimental garden (Yagihashi et al. 1999; 2000).

In a previous study (Amodeo and Zalba 2013) we described the age structure of invasive stands of *P. mahaleb* in this area, and proposed a lag phase of 8–18 years between the establishment of the founder tree and local effective recruitment due to an increment in disperser attendance associated with the increase in fruit offer. We now suggest a complementary scenario in which the colonization might include a facilitation process between the sex morphs, with interesting consequences for the establishment and growth of new invasion foci. As mentioned before, gynodioecious species can show Allee effects associated with mate finding and pollen limitation that may affect their ability to colonize and spread. These types of interactions have been highlighted as important factors affecting invasion processes (McCormick et al. 2010). Based on the variability observed in fruit set and the results reported within the species native range, we propose that the homogeneity in reproductive success between females and hermaphrodites could be a relatively recent situation. The initial stages of invasion, characterized by low population densities, seem to be more favourable for self-pollinating individuals, whose spread would create better conditions for obligate out-crossers. The progressive increase in the abundance of the latter could affect the sex ratio and the dynamics of local invasions, including initial lags in spread. This scenario might be tested in future studies focused on the relative importance of sex morphs in gynodioecious populations at early stages of invasion.

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Tables

Table 1. Descriptive parameters of the studied *Prunus mahaleb* populations in the Ernesto Tornquist Provincial Park, Argentina

Population	Understory		Open grasslands	
	1	2	3	4
Total density of <i>P. mahaleb</i> (ind./ha)	237.2	216.8	281.5	154.7
<i>P. mahaleb</i> <4 years old (%)	62.9	56.6	71.9	66.2
<i>P. mahaleb</i> 4-10 years old (%)	30.0	38.1	14.8	22.2
<i>P. mahaleb</i> >10 years old (%)	7.1	5.3	13.3	11.6
Other trees > 10 m height (ind./ha)	77.6	88.0	-	5.5
Other trees < 10 m height (ind./ha)	9.9	6.5	18.4	31.8
Percentage of females (%)	2.6	4.8	11.6	1.8

Table 2. Likelihood Ratio Test for the Generalized Linear Models analysing the relationship of flower production, fruit production and fruit set with respect to the basal stem diameter (*BSD*) and sex morph of *Prunus mahaleb* individuals growing at the Ernesto Tornquist Provincial Park, Argentina.

Response	Model	2010-2011 (n=41)			2011-2012 (n=13)			2012-2013 (n=20)		
		LRT	d.f.	p*	LRT	d.f.	p*	LRT	d.f.	p*
variable										
<i>Flower</i>	<i>Log(BSD)</i>	117.758	40	<0.001	-	-	-	55.83	19	<0.001
<i>production</i>	<i>Log(BSD)+Gender</i>	1.750	39	0.19	-	-	-	1.94	18	0.16
	<i>Log(BSD)*Gender</i>	0.216	38	0.64	-	-	-	1.25	17	0.26
<i>Fruit</i>	<i>Log(BSD)</i>	75.354	40	<0.001	29.5	12	<0.001	69.56	19	<0.001
<i>production</i>	<i>Log(BSD)+Gender</i>	0.006	39	0.94	0.52	11	0.47	1.83	18	0.18
	<i>Log(BSD)*Gender</i>	0.925	38	0.34	0.6	10	0.44	4.27	17	0.04
<i>Fruit set</i>	<i>Log(BSD)</i>	2664.4	40	<0.001	-	-	-	3246.5	19	<0.001
	<i>Log(BSD)+Gender</i>	458.47	39	<0.001	-	-	-	20323.0	18	<0.001
	<i>Log(BSD)*Gender</i>	2114.27	38	<0.001	-	-	-	1036.1	17	<0.001

* The comparison between alternative models was performed adding the terms sequentially in the order shown.

Denominator degrees of freedom are shown.

Table 3. Likelihood Ratio Test for the Generalized Linear Models analysing the relationship of fruit size, stone size and germination response with respect to the basal stem diameter (*BSD*) and the sex morph of *Prunus mahaleb* individuals growing at the Ernesto Tornquist Provincial Park, Argentina.

Response variable	Model	2010-2011		
		LRT	d.f.	p*
<i>Fruit size</i>	<i>Log(BSD)</i>	0.308	22	<0.001
	<i>Log(BSD)+Gender</i>	0.029	21	0.29
	<i>Log(BSD)*Gender</i>	0.063	20	0.12
<i>Stone size</i>	<i>Log(BSD)</i>	0.045	21	0.12
	<i>Log(BSD)+Gender</i>	0.022	20	0.27
	<i>Log(BSD)*Gender</i>	0.0002	19	0.90
<i>Germination (laboratory)</i>	<i>BSD</i>	143.65	20	<0.001
	<i>BSD+Gender</i>	0.17	19	0.68
	<i>BSD*Gender</i>	11.98	18	<0.001
<i>Germination (experimental garden)</i>	<i>BSD</i>	16.78	34	0.42
	<i>BSD+Gender</i>	16.77	33	0.94
	<i>BSD*Gender</i>	16.53	32	0.62

*The comparison between alternative models was performed adding the terms sequentially in the order shown. The analyses of fruit and stone weight showed similar results as the volume and are not shown. Denominator degrees of freedom are shown.

Figure captions

Figure 1 Flowers of hermaphrodites (a, b) and females (c, d) in *Prunus mahaleb* populations growing at the Ernesto Tornquist Provincial Park, Argentina. Arrows indicate globose anthers filled with pollen of hermaphrodites and shrunken anthers with no pollen of females

Figure 2 Relationship between the total number of fruits produced per plant and its basal stem diameter (BSD) for hermaphroditic and female *Prunus mahaleb* individuals growing at the Ernesto Tornquist Provincial Park, Argentina. Black lines indicate the fitted values of Generalized Linear Models and grey area indicates the 95% confidence interval

Figure 3 Relationship between fruit set and basal stem diameter for hermaphrodite and female *Prunus mahaleb* individuals growing at the Ernesto Tornquist Provincial Park, Argentina. Lines indicate the fitted values of Generalized Linear Models and grey area indicates the 95% confidence interval

Figure 4 Germination rate and non-germinated seeds viability of *Prunus mahaleb* seeds collected from hermaphroditic and female plants of different categories of basal stem diameter (BSD) growing at the Ernesto Tornquist Provincial Park, Argentina. Similar letters indicate no significant differences according to pairwise Tukey tests ($p=0.05$)

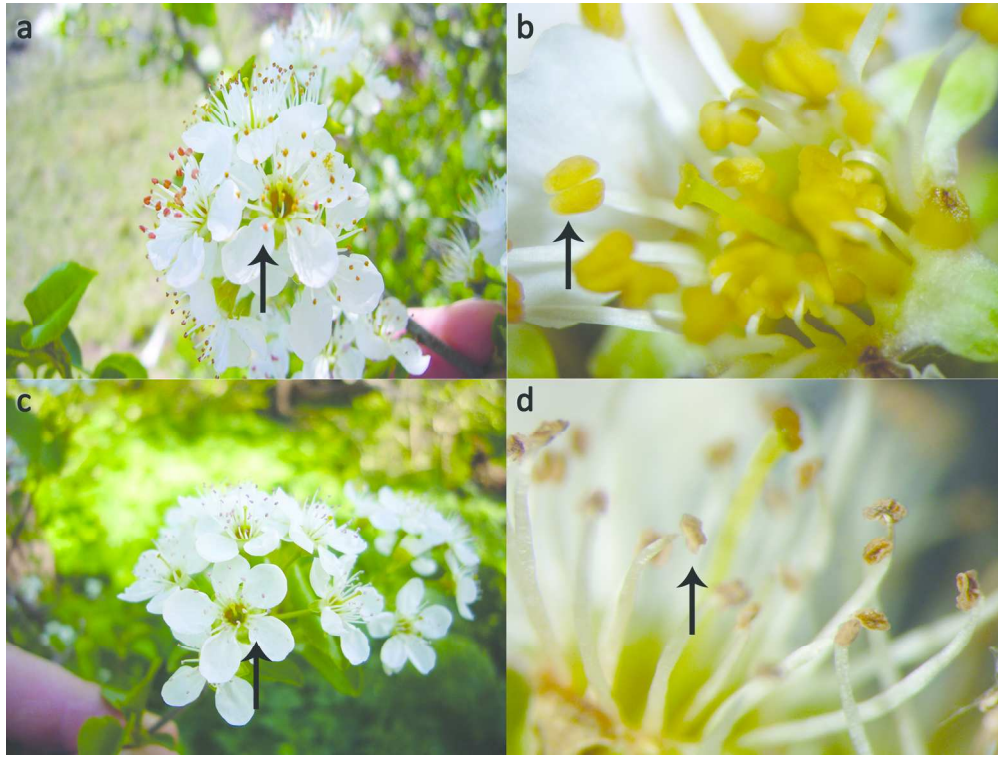


Figure 1 Flowers of hermaphrodites (a, b) and females (c, d) in *Prunus mahaleb* populations growing at the Ernesto Tornquist Provincial Park, Argentina. Arrows indicate globose anthers filled with pollen of hermaphrodites and shrunken anthers with no pollen of females

199x150mm (300 x 300 DPI)

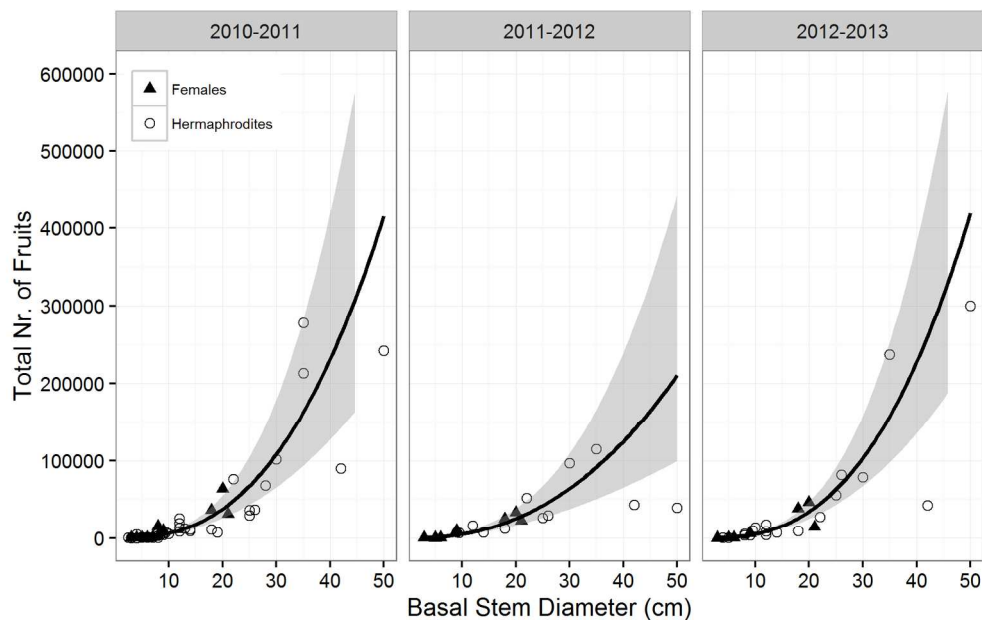


Figure 2 Relationship between the total number of fruits produced per plant and its basal stem diameter (BSD) for hermaphroditic and female *Prunus mahaleb* individuals growing at the Ernesto Tornquist Provincial Park, Argentina. Black lines indicate the fitted values of Generalized Linear Models and grey area indicates the 95% confidence interval

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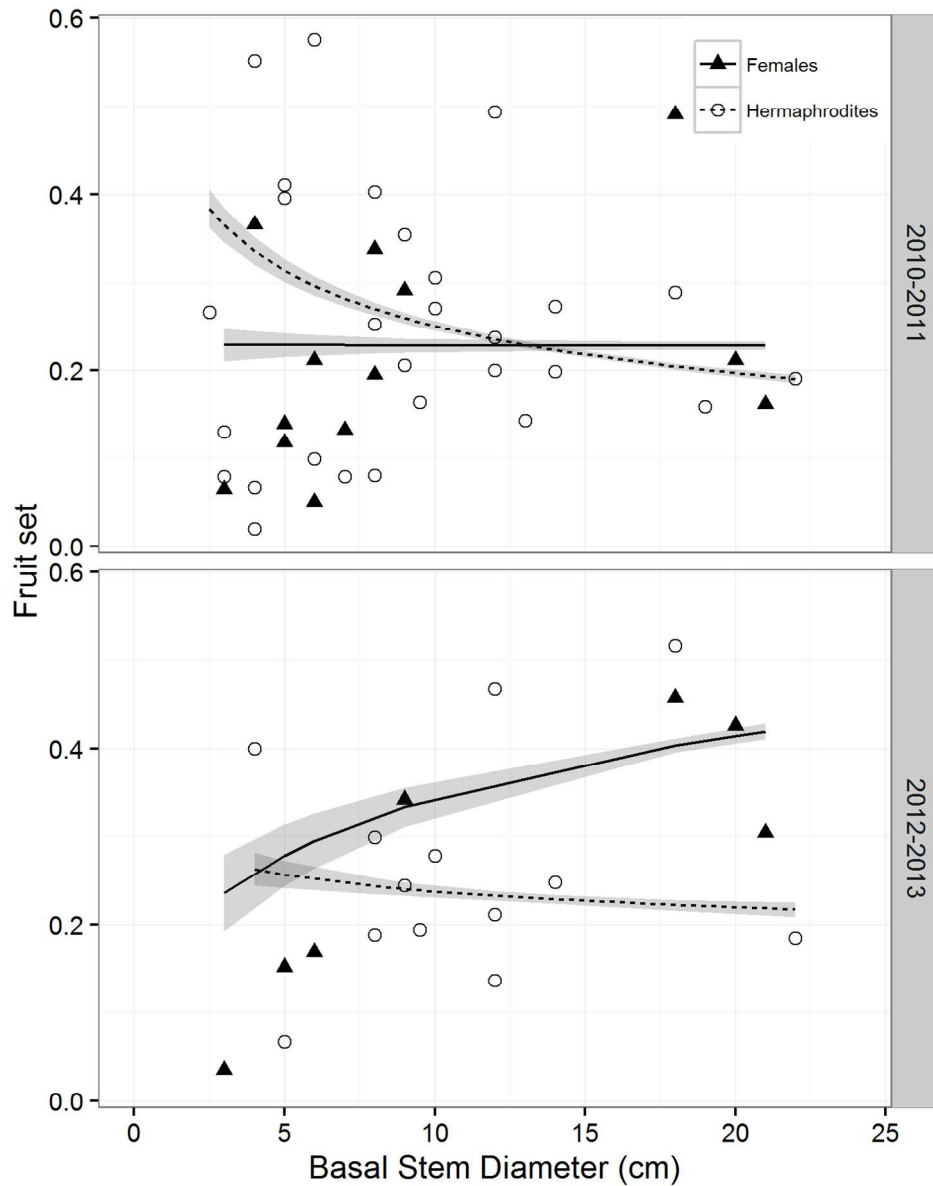


Figure 3 Relationship between fruit set and basal stem diameter for hermaphrodite and female *Prunus mahaleb* individuals growing at the Ernesto Tornquist Provincial Park, Argentina. Lines indicate the fitted values of Generalized Linear Models and grey area indicates the 95% confidence interval

128x159mm (300 x 300 DPI)

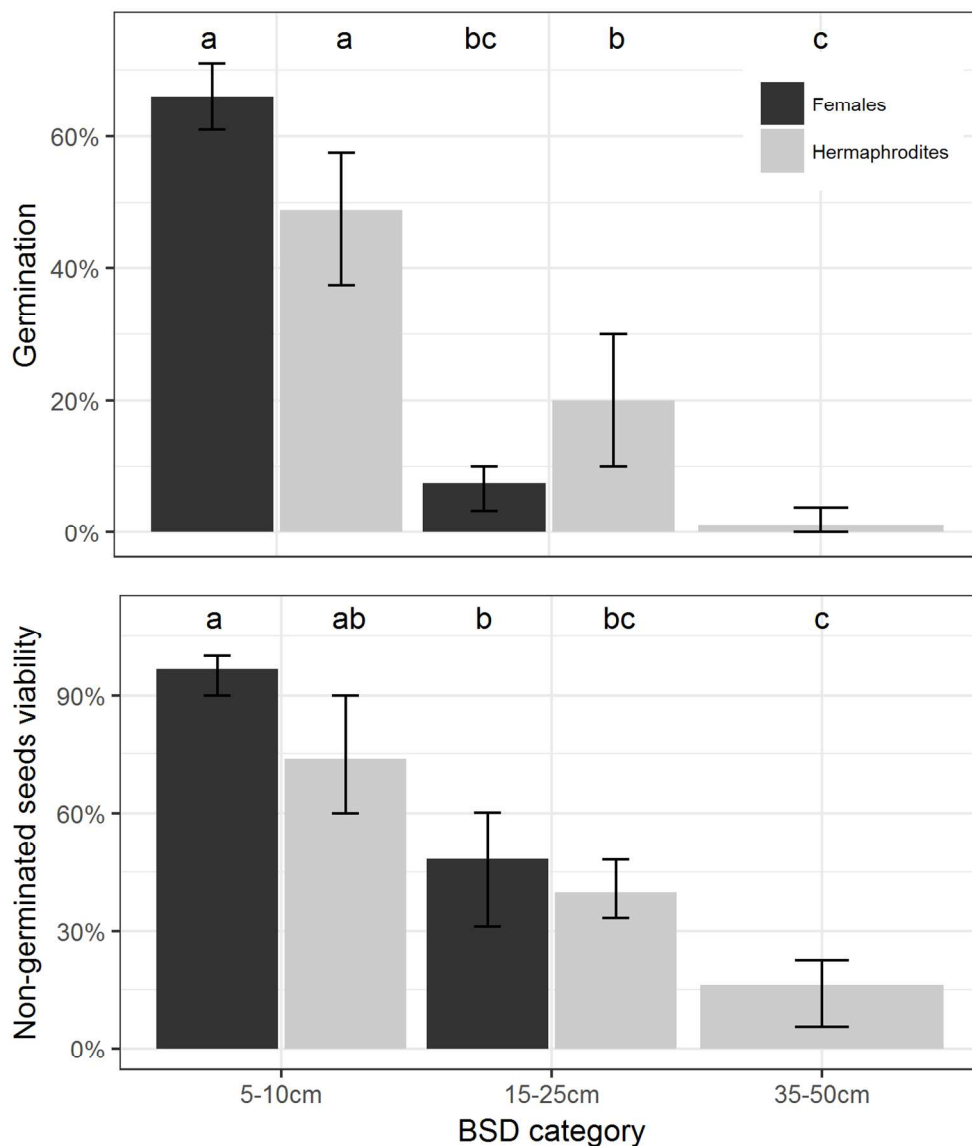


Figure 4 Germination rate and non-germinated seeds viability of *Prunus mahaleb* seeds collected from hermaphroditic and female plants of different categories of basal stem diameter (BSD) growing at the Ernesto Tornquist Provincial Park, Argentina. Similar letters indicate no significant differences according to pairwise Tukey tests ($p=0.05$)

128x149mm (300 x 300 DPI)