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Functional dioecy in *Gleditsia amorphoides* (Fabaceae)

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Abstract. The genus *Gleditsia* (Fabaceae) comprises woody shrubs and trees that grow in temperate and subtropical regions around the world. This genus is characterised by sexual polymorphism and functionally unisexual flowers. *Gleditsia amorphoides* is the southernmost species of the genus, and is widely used as a source of timber and derived products for industrial applications (galactomannans are extracted from its seeds and saponins are derived from its fruits). The species is endemic to the Chaquenean Forest of South America. It is described as morphologically androdioecious, with male and perfect flowers appearing on different plants. In the current study, we characterised floral morphology, experimentally tested the breeding system and analysed flower visitors. Results indicated that *G. amorphoides* staminate flowers produce viable pollen grains and that perfect flowers have a functional gynoecium and empty anthers, where pollen abortion occurs early in floral development. The species relies on outcrossing, which depends mainly on pollen carried by insect pollinators, to produce seeds and fruits. We conclude that *G. amorphoides* is functionally dioecious, with staminate and pistillate floral morphs.

Additional keywords: Caesalpinioideae, Chaquenean Forest, plant reproduction, sexual polymorphism, unisexuality.

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Introduction

Gleditsia L. (Fabaceae) is a genus composed primarily of thorny trees and shrubs from temperate and subtropical regions of the world. The genus comprises 10–30 specific taxa, as proposed by the Missouri Botanical Garden (2017) Tropicos® classification system and the taxonomy of Gordon (1966). According to the Missouri Botanical Garden (2017), most of these species (*Gleditsia australis* Hemsl., *Gleditsia fera* (Lour.) Merr., *Gleditsia japonica* Miq., *Gleditsia microphylla* D.A.Gordon ex Y.T.Lee, *Gleditsia sinensis* Lam.) are from different regions of Asia (China, Pakistan, Japan, North and South Korea and India); three taxa (*Gleditsia aquatica* Marshall, *Gleditsia* x. *texana* Sarg., and *Gleditsia triacanthos* L.) are from North America (Mexico and the USA); and just one species (*Gleditsia amorphoides* [Griseb.] Taub.) is endemic to South American countries (Brazil, Paraguay, Uruguay and Argentina).

At the southernmost limit of the distribution of the genus, *Gleditsia amorphoides* (Griseb.) Taub. (basionym *Garugandra amorphoides* Griseb.) grows wild in southern Brazil, Paraguay, Uruguay and northern Argentina (Missouri Botanical Garden 2017). Phylogenetic analysis (Schnabel and Wendel 1998) has shown that this species forms a clade with two eastern Asian species (*G. sinensis* and *G. fera*); however, the origin of this Asian–South American disjunction within the genus is still poorly understood (Gordon 1966; Raven and Polhill 1981; Schnabel and Wendel 1998). *Gleditsia amorphoides* is a source of timber (Tortorelli 2009) and products derived from various plant parts for industrial applications. Galactomannans extracted from its seeds are currently used in the food industry (Rothman and Rique 1959; Perduca *et al.* 2013), and saponins derived from its fruits have been proposed as adjuvants to agrochemicals (Prola 2011).

Studies on the reproductive biology of *Gleditsia* indicate to sexual polymorphism, unisexuality and widely diverse mating system as its distinctive features (Gordon 1966; Schnabel and Hamrick 1990; Tucker 1991). Two species in the genus, namely *G. triacanthos* and *G. sinensis*, have been cited as andromonoecious, i.e. they have a mixture of staminate and perfect flowers (Kalin Arroyo 1981). The first has also been described as mostly dioecious (Schnabel and Hamrick 1990), even with populations including monoecious, male and polygamous trees (Gordon 1966). Flowers produced by various species of

the *Gleditsia* genus can be structurally perfect or unisexual (Tucker 1991). However, it is common for perfect flowers to acquire unisexual functionality in the course of floral ontogeny (Tucker 1991).

For *G. amorphoides*, botanical descriptions mention the presence of morphologically perfect flowers and staminate flowers on different plants (Burkart *et al.* 1987; Ulibarri 1997), a sexual system known as androdioecy (Richards 1986). Unfortunately, as with their congeners, these studies were purely descriptive and were not experimental. No detailed experimental studies on the reproductive biology of this particular species of locust tree have been undertaken.

A functional interpretation of gender in androdioecious populations is imperative to correctly define plant sexuality, because the hermaphrodite morph generally functions as a male parent (Barrett 2002). Therefore, many morphologically androdioecious species are functionally dioecious (Mayer and Charlesworth 1991). Male sterility in perfect flowers can be associated with atrophied anthers, pollen abortion, inaperturate pollen and low pollen germination (Anderson and Symon 1989; Hoffman 1992; Lepart and Dommée 1992; Knapp *et al.* 1998; Strittmatter *et al.* 2006; Sun *et al.* 2009). Thus, the objective of the current work was to evaluate the physiological functionality of the reproductive system of *G. amorphoides*. To this end, we characterised morphology and flower visitors to floral morphs, and determined the mating system experimentally rather than by observation alone.

Materials and methods

Study area

Samples were collected and field experiments were performed during the months of September and October in 2012 and 2014 in two populations of *G. amorphoides* located at Los Tábanos (28°27′33″S, 59°59′02″W) and Las Claritas (28°33′32.45″S, 59°33′55.83″W) in Santa Fe Province, Argentina. These populations are located in the Bosques Altos area, which is part of the Cuña Boscosa region of the Chaquenean phytogeographic province. Both occupy higher elevations (up to 50 m above sea level) and have a high level of intraspecies diversity (Lewis 1981; Pensiero *et al.* 2005). The trees at Los Tábanos belong to a commercial plantation for the production of seed used to make flour. The individuals we examined consisted of 15 trees with morphologically male flowers and 15 trees with morphologically perfect flowers. The trees were ~20 years old at the time of the study and originally came from seeds collected from wild populations from surrounding indigenous forests. The *G. amorphoides* stand at Las Claritas is a wild, uncultivated population. In an attempt to duplicate methodology as closely as possible, we evaluated 20 individuals, 10 with male flowers and 10 with perfect flowers, at the second study site, Las Claritas. One herbarium voucher per population was deposited at the Herbarium SF of the Facultad de Ciencias Agrarias de la Universidad Nacional del Litoral, Santa Fe, Argentina (voucher code for Los Tábanos: C325; voucher code for Las Claritas: C330).

General morphology of test populations

Individuals of *G. amorphoides* are deciduous trees that usually grow to a mature height of 3–10 m, with some individuals reaching up to 20 m in height. Branches have single or ramified spines. Leaves are often on short shoots; herbaceous leaflets are arranged in groups of 6–15 pairs per composite leaf, and leaflets range in size from 1.0–4.0 cm long × 0.14–7.0 cm wide (Ulibarri 1997). Inflorescences are cymose panicles, also called pseudoracemes (Tucker 1991). Flowers are white–greenish, briefly pedicellate, and present at 6–7 mm in diameter; each flower has three to six sepals and petals. Staminate flowers have five or six stamens. Perfect flowers are multi-ovulated, with a sessile or subsessile ovary and an expanded stigma, and have three to six staminoids. Pods are glabrous, falcate, woody, and appear blackish in colour. Typically, legumes are 4.0–10.0 cm long × 2.5–5.0 cm wide (Ulibarri 1997).

Floral morphology

In the present study, floral morphology was quantified in a total of 80 flowers ($n = 20$ flowers from each of two floral morphs present in the two populations). During the 2012 reproductive season, flowers were randomly chosen from an individual tree and were subsequently fixed in formalin–acetic acid–ethanol (FAA) prepared in a 1 : 1 : 3 ratio. The following measurements were recorded for each flower: length of flower (receptacle + calyx and corolla); mean number and length of stamens and staminoids; number of ovules; and number of pollen grains. Colour of flowers and presence of nectar were subjectively determined by direct field observations. Pollen viability was evaluated by observations of pollen-tube growth at 12 and 24 h after floral anthesis and hand-pollination of perfect floral morphs. In addition, we counted the number of flowers in three compound inflorescences of each floral morph in five trees per population ($n = 30$ inflorescences per two populations). Floral dehiscence was evaluated by direct observation.

To test for pollen grains in anthers of stamens and staminoids, staminate and perfect flowers were fixed in FAA and stored in 70% ethanol. Dissected material was dehydrated in an alcohol–xylol series and was subsequently embedded in paraffin wax (Zarlavsky 2014). Sections were cut with a rotary microtome (Reichert Technologies, Austria) at 9-μm thickness and were stained with safranin and fast green (Zarlavsky 2014).

Floral visitors

Foraging behaviour and duration of visits to flowers were studied during 15-min observation periods. Observations were conducted every 2 h, between 0800 hours and 1800 hours, over a period of four non-consecutive days at Las Claritas (total observation time: 360 min). Observations were made only when weather conditions allowed moderate to high insect activity (temperature >15°C, null or moderate wind, sunny days). Observations were conducted in 2012 and again in 2014 during peak flowering period (September and October). We used entomological nets to capture insects when they were foraging on flowers. Insects were then killed *in situ* and were preserved for subsequent identification. Pollen load on floral visitors was estimated under a binocular microscope (Olympus CH30, Olympus Optical Co., Ltd., Japan; ×100 magnification); pollen grain identification was facilitated by passing a block of gelatin–glycerin infused with safranin over the bodies of

insects to extract the pollen grains adhered to them. Insect identification was performed to the lowest possible taxonomic level. All captured specimens were preserved in the entomological collection of the Department of General Botany, Facultad de Agronomía, Universidad de Buenos Aires (UBA).

Breeding system

During peak flowering period of the 2014 reproductive season, pollination experiments were conducted at Los Tábanos. The following four treatments were performed *in situ* on a known number of perfect virgin (unpollinated) flowers that were buds near dehiscence at the time of the treatment: (1) an open-pollination (control) treatment, in which flowers were not manipulated and were exposed to free pollination; (2) a hand-cross-pollination (xenogamy) treatment, in which buds were covered with voile bags, which excluded wind pollination and floral visitors, and were subsequently hand-pollinated using a mix of pollen from several individuals (flowers were voile-bagged post-pollination until fruit set); (3) a floral-visitor exclusion treatment equating to wind pollination, in which buds were bagged with a 1-mm mesh until fruit set, excluding floral visitors and allowing the passage of the airborne pollen; and (4) an apomixis (asexual seed formation) treatment to evaluate the production of seed without egg fertilisation, in which flowers were voile-bagged until fruit set.

Table 1. Percentage of fruit set and seed set (mean ± s.d.) under different treatment regimens used to elucidate the breeding system of *Gleditsia amorphoides*

n, total number of treated flowers per treatment. –, no seed set was possible because no fruit was formed. Different letters within a column indicate a significant difference by Di Rienzo, Guzmán and Casanoves (DGC) test (at $P = 0.05$)

Treatment	*n*	Fruit set (%)	Seed set (%)
Open-pollination	849	8 ± 1a	85 ± 3a
Hand-cross-pollination	500	11 ± 2a	79 ± 2a
Anemophily	464	3 ± 1b	25 ± 9b
Apomixis	492	0 ± 0c	–

Fig. 1. *Gleditsia amorphoides* flowers. (*a*) Female inflorescence, and (*b*) female flower. (*c*) Male inflorescence and (*d*) male flower. Note that images are presented at different scales for clarity. Scale bars = 5 mm.

Table 2. Morphometry of male and female flowers (mean ± s.d.) of two populations of *Gleditsia amorphoides* located at Los Tábanos (LT) and Las Claritas (LC) in Santa Fe Province, Argentina

Different letters indicate significant difference between male and female averages by Di Rienzo, Guzmán and Casanoves (DGC) test (at $P = 0.05$). –, none present

Parameter	Male flower (mm)			Female flower (mm)		
	LT	LC	Average	Average	LC	LT
Flower length	5.6 ± 0.4	5.7 ± 0.5	5.6 ± 0.5b	7.6 ± 1.2a	7.8 ± 1.4	7.4 ± 1.1
Number of stamens/staminoids	8.0 ± 1.0	8.0 ± 2.0	8.0 ± 1.0a	4.0 ± 0.5b	3.9 ± 0.6	4.0 ± 0.5
Length of stamens/staminoids	4.0 ± 0.3	5.7 ± 0.8	4.9 ± 1.1a	4.2 ± 1.8a	5.8 ± 0.8	2.6 ± 0.6
Number of ovules	–	–	–	8.0 ± 1.0	8.0 ± 1.0	8.0 ± 1.0

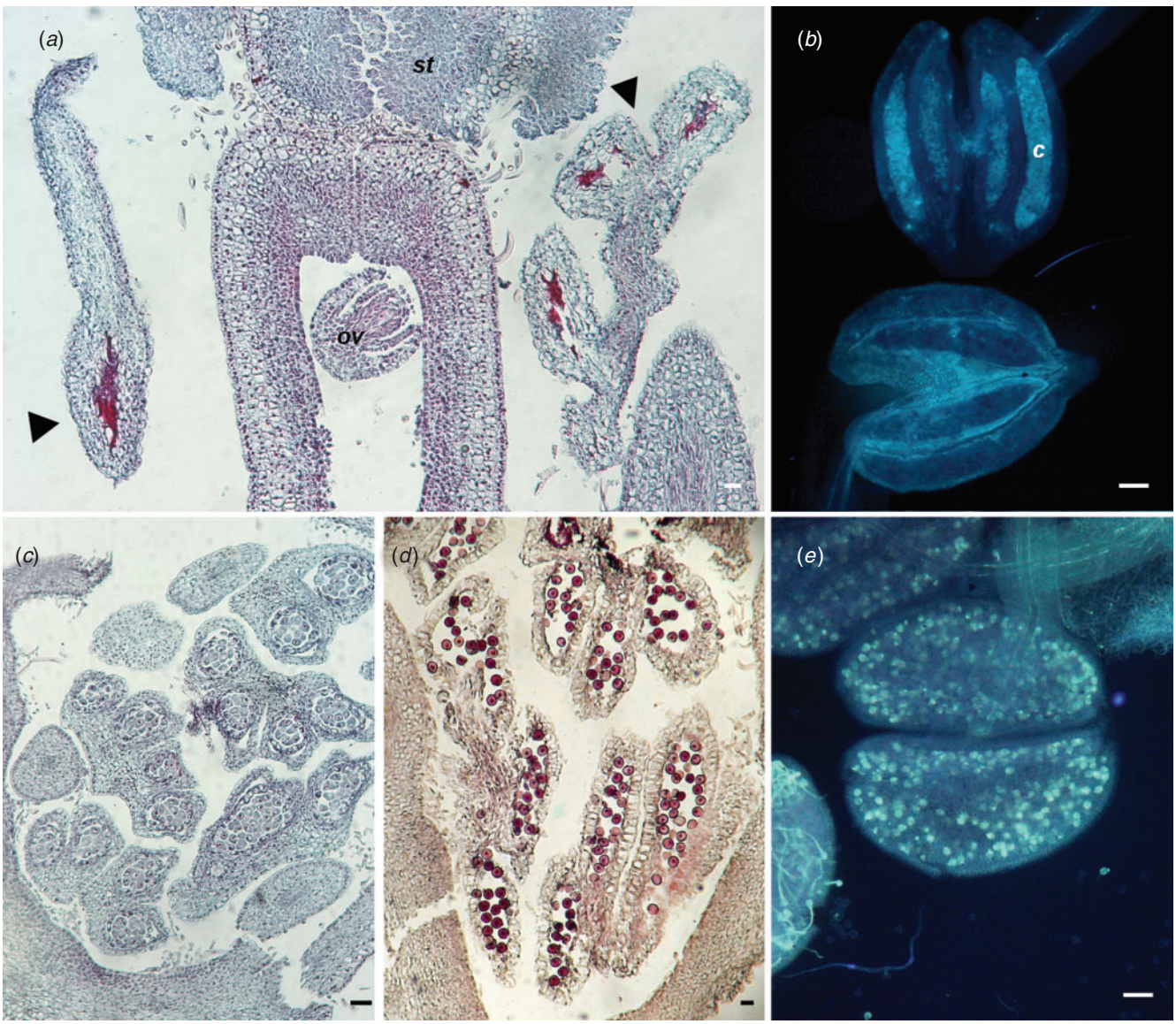

Fig. 2. Photomicrographs of (*a*, *b*) perfect and (*c*–*e*) staminate flowers of *Gleditsia amorphoides*. (*a*) Longitudinal section of the ovary and staminoids (black arrows); (*b*) empty anthers. Transverse section of anthers in functional stamens, including (*c*) microsporangial tissue and (*d*) pollen grains; (*e*) anthers with pollen grains. c, callose; ov, ovule; st, stigma. Note that images are presented at different scales for clarity. Scale bars = 0.1 mm.

Hand-cross-pollination (xenogamy) treatments were performed once, when stigma were receptive (10–24 h post-anthesis), by brushing fresh pollen across a stigma until the deposition of pollen grains was visible to the human eye. Stigmatic receptivity was evaluated by observation of the pollen-tube growth after hand-pollinations. For this, two randomly chosen subsamples of 10 flowers each from five trees ($n = 20$ flowers total) were hand-pollinated at 10 and 24 h after anther dehiscence. Exactly 24 h after hand-pollination, the flowers were picked, fixed in FAA and stained with aniline blue (Zarlavsky 2014). Floral tissue was observed under a fluorescence microscope (Leica DM1000, Leica Microsystems GmbH, Wetzlar, Germany), and photographs were taken with a Canon EOS RebelT2i (DS126271) camera (Canon Mexicana, S. de R.L. de C.V., México DF).

Seed set (percentage of full seeds in relation to ovule number) and fruit set (percentage of fruits formed in relation to initial flowers) were variables used to compare the four treatments. The number of ovules per flower was obtained by averaging ovules counted per flower, as per the 'Materials and methods: floral morphology' section.

Statistical analysis

Morphological variables

Normality and homogeneity of variances were tested first. Then, differences in morphological variables of staminate versus (vs) perfect flowers, were evaluated by applying a Student's *t*-test to independent samples. The same statistical test was used to quantify differences between the number of flowers per inflorescence found in morphologically male and perfect inflorescences. We used the 2011 version of InfoStat software to run statistical analyses (Di Rienzo *et al.* 2011).

Experimental treatments

Treatments were applied in a randomised, complete-block design with 12 replicates (where the replicate was an individual, female tree). This design was utilised to maximise replicates so as to avoid any possible between-tree effects on fruit and seed set. Within each replicate, each of the four treatments was repeated four times; the experimental unit was an inflorescence at a height of ~1.5–2 m above ground level in the four cardinal positions, north, south, east and west. Each inflorescence could be considered as a subplot, or as a pseudo-replicate. In total, 48 inflorescences per treatment were analysed. The total number of flowers included in each treatment is shown in Table 1. To minimise the experimental error, non-emasculated small flowers and previously opened flowers were removed from selected inflorescences, and, hence, were not included in the analysis.

Differences in seed and fruit set between treatments were analysed by the adjustment of general linear models (GLM), in conjunction with the linear mixed-effects (*lme*) function of the non-linear mixed-effects (*nlme*) models (Pinheiro *et al.* 2011), and by using the R statistical language (R Core Team 2011). The GLM for this experiment comprised a fixed effect for treatments and two random effects (complete blocks and experimental units nested within complete blocks). We modelled the error structure considering the following four items: (1) independence of errors; (2) compound symmetry (using the corCompSymm function); (3) unrestricted correlations structure (using the corSymm function); and (4) autoregressive Order 1 (using the corCAR1 function). The best model was the independent error structure, according to the minimisation of the Akaike information criterion and Bayesian information criterion.

We used the 2011 interface provided by InfoStat for the analyses (Di Rienzo *et al.* 2011). Means of treatments were compared using the Di Rienzo, Guzmán and Casanoves test (Di Rienzo *et al.* 2002). Normality and homoscedasticity were tested graphically (using the Q–Q and the residual vs predictor plots respectively). Variance structure was modelled according to the *varIdent* function for seed-set variables and the *varExp* function for fruit-set variables; both functions are part of the *nlme* package of R (Pinheiro *et al.* 2011).

Results

Floral morphology

Staminate and perfect flowers were similar in appearance, with a green calyx and corolla (Fig. 1); however, they differed in floral size and number of flowers per inflorescence (Table 2). Staminate flowers were smaller than the perfect ones (5.63 ± 0.46 mm long vs 7.60 ± 1.23 mm long respectively; $t = 6.73$; $P < 0.0001$), and staminate inflorescences had more flowers per inflorescence than the perfect ones (45 ± 16 male flowers per inflorescence vs 16 ± 7 perfect flowers per inflorescence; $t = 9.15$; $P < 0.0001$). Functional stamens (8 ± 1 stamens), with $14\,700 \pm 3623$ pollen grains per flower, were observed only in male flowers (Fig. 2*c–e*). In female flowers, 4 ± 1 staminoids that did not produce pollen were observed (Fig. 2*b*). In these flowers, suppression of microsporogenous tissue was evident from the start of their development (Fig. 2*a*).

Table 3. Flower visitors captured on male and female individuals of *Gleditsia amorphoides* at Los Tábanos, Santa Fe Province, Argentina
T, total number of individuals captured

Order	Family	Species	T
Coleoptera	Cerambycidae	Morphospecies 1	1
	Lampyridae	Morphospecies 2	1
	Scarabaeidae	*Gymnetis hebraica* Drapiez	1
		Morphospecies 3	1
Diptera	Calliphoridae	*Chrysomya chloropyga* Wiedemann	1
	Syrphidae	*Allograpta* sp. 1	1
		Copestylum sp. 1	1
		Palpada distinguenda Wiedemann	2
		Palpada elegans Blanchard	1
		Palpada rufiventris Macquart	4
		Quichuana sp. 1	2
	Tabanidae	Morphospecies 4	1
	Tachinidae	Tachinidae sp. 1	1
Hymenoptera	Andrenidae	*Psaenythia* sp. 1	1
	Apidae	*Apis mellifera* Linnaeus	17
	Formicidae	Formicidae sp. 1	2
		Formicidae sp. 2	2
	Halictidae	*Augochloropsis* sp. 1	1
	Vespidae	*Polybia occidentalis* Olivier	1
		Polybia sericea Olivier	7

A functional ovary with 8 ± 1 ovules was observed only in perfect flowers (Fig. 2*a*). Ovary formation was not observed at any stage of male-flower development (Fig. 2*c*, *d*). Both floral morphs presented nectar exposed as droplets on the inner surface of the receptacle. In both 2012 and 2014, no variations were observed in the sexual expression of either floral morph in studied populations.

Floral visitors

In total, 49 insects belonging to the families Coleoptera (four species), Diptera (nine species), and Hymenoptera (seven species) were captured (Table 3, Fig. 3). *Apis mellifera* was the most abundant. This species, together with individuals of the family Syrphidae, were the most frequent floral visitors to male and female flowers. The visitation rate was similar in both floral morphs.

All floral visitors captured carried pollen grains of *G. amorphoides* on their bodies in great numbers, to the flower ovules. We observed 501–1300 pollen grains on 46% of individual insects, 101–500 pollen grains on 33% of insects, and 50–100 pollen grains on 21% of the floral visitors. Pure pollen load was observed adhering to 92% of the insects.

Breeding system

Observations of pollen-tube growth after hand-pollination showed that pollen tubes had entered into the ovules in all evaluated flowers (Fig. 4). Seed and fruit set varied between treatments ($r^2 = 0.96$, $F = 738.74$, $P < 0.0001$ for seed set; and $r^2 = 0.41$, $F = 17.31$, $P < 0.0001$ for fruit set) (Table 1). The best seed set was obtained under hand-cross- and open-pollination treatments (79% and 85% seed set respectively). A similar result occurred with fruit set, with 11% and 8% fruit set occurring under hand-cross- and open-pollination treatments respectively. No fruit set under the apomixis treatment was observed. Low fruit set (3%) was noted in the treatment equating to wind pollination.

Discussion

Our results showed that *G. amorphoides* is a xenogamous species that is highly dependent on insects for pollination and the

Fig. 3. Some floral visitors to *Gleditsia amorphoides*. (*a*) An undertermined species of Formicidae, (*b*) the bee *Psaenythia* sp., (*c*) an undetermined species of Tachinidae fly and (*d*) *Apis mellifera*. Note that images are presented at different scales for clarity. Scale bars = 5 mm.

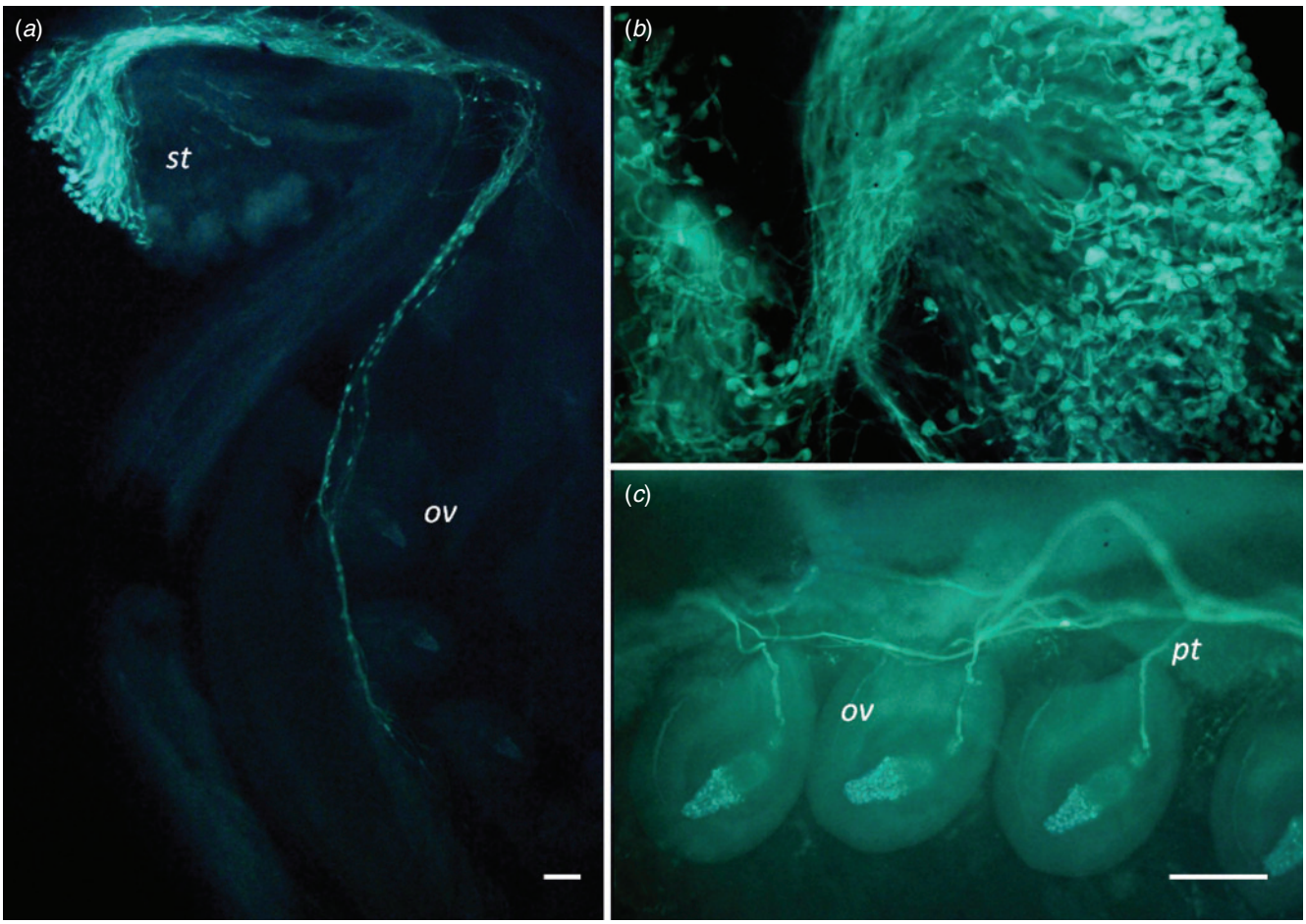

Fig. 4. Pollen-tube growth in open-pollinated flowers of *Gleditsia amorphoides*. (*a*) Pollen-tube growth across stigma and ovary 24 h after floral anthesis; (*b*) germinated pollen grains in stigma tissue 24 h after floral anthesis; (*c*) ovule fertilisation. h, hours; ov, ovule; pt, pollen tube; st, stigma. Note that images are presented at different scales for clarity. Scale bars = 0.5 mm.

formation of seeds and fruits. In our current work, both direct observation and experimental treatments showed that the two studied populations were functionally dioecious, with two functionally unisexual morphs. The female morph had a functional gynoecium that formed viable seeds and atrophied anthers that, from the beginning of their development, failed to form pollen. The male morph had only staminate flowers, with functional pollen grains but without gynoecia. As is typical of dioecious species (Lloyd and Webb 1977; Barrett and Hough 2013), the differences in primary sexual characteristics (the androecium and gynoecium) of *G. amorphoides* are reinforced by secondary sexual dimorphism in some floral traits. In this sense, the males we observed bloomed earlier (direct observation, data not shown) and produced smaller flowers, with more flowers per inflorescence than in females.

Changes in patterns of floral ontogenetic development processes are common in subfamily Caesalpinioideae and are usually due to organ suppression, or conversion (Tucker 1987, 1988, 2000, 2003; Hokche and Ramirez 1990; Bruneau *et al.* 2014). In *G. amorphoides*, floral buds of both sexual morphs initiate both stamen and carpel primordia, but reproductive organs are suppressed selectively to produce either male or female flowers (Tucker 2003). As proposed for the genus, unisexuality in floral morphs of *G. amorphoides* could be attributed to organ suppression. In the *G. amorphoides* populations that we observed, unisexuality in female flowers was associated with suppression of microsporogenous tissue from the start of development. In male flowers, ovarian differentiation was not observed at any stage of male flower development. This would suggest that ovary formation does not occur, or is suppressed at very early stages of development. For other species of the genus, female sterility has been associated with the development of ovules that are much smaller than usual (Tucker 1991).

The presence of staminoids in our test populations could be the consequence of a recent evolutionary move towards dimorphism, which is a component of incomplete sexual differentiation (Charlesworth 1984; Strittmatter *et al.* 2002). Many functionally dioecious species, with females retaining substantial anther vestiges, are considered arguments for dioecy evolution from hermaphroditism, via androdioecy (Charlesworth 1984; Charlesworth and Guttman 1999). In these sense, androdioecy in *G. amorphoides* could be an intermediate step in the evolution of dioecy through the spread of a female-sterility mutation in initial hermaphrodite populations. It has been proposed that the most favourable situation for the spread

of a female-sterility mutation is supported by (1) complete outcrossing, with very low selfing and high inbreeding depression (Lloyd 1975; Wolf and Takebayashi 2004) and (2) male mutants with at least double the fitness of hermaphrodites (Lloyd 1975). A higher male fitness, related to more flowers and inflorescences in male individuals than in hermaphroditic ones, has been observed in other morphologically androdioecious and functionality subdioecious species (Obeso 2002; Verdú 2004; Verdú *et al.* 2004). The same situation was observed in the present study. Males of *G. amorphoides* had more stamens per flower and flowers per inflorescence than did co-sexuals. This condition could have allowed the invasion of an ancestral hermaphrodite population by males, in a possible route to dioecy via androdioecy.

Monoecy has been proposed as one of the main routes in the evolution of numerous dioecious species (Ainsworth 2000; Barrett 2002). For species that have developed dioecy through this evolutionary route, it was predicted that individuals should show some sex lability under certain environmental conditions, producing flowers of the 'opposite sex' (Freeman *et al.* 1997). In the same labile systems, androdioecy, gynodioecy and subdioecy are included (Ainsworth 2000). Sexual expression in flowers of *Gleditsia* species is an unstable trait susceptible to change or transformation throughout floral development (Tucker 1991). In fact, it has been noted for *G. triacanthos* that trees are either staminate or fruit-forming and the possibility of a change in sex from one year to the next has been suggested (in Tucker 1991). For *G. amorphoides*, occasional presence of fruits on males could also represent a condition of sex lability. In this context, it is possible that species of *Gleditsia* have monoecious ancestors.

Conclusions

The present study demonstrated experimentally that *G. amorphoides* is a functionally dioecious species, with sexual dimorphism of floral traits. However, additional studies should be conducted to understand both the selective forces that might favour the retention of stamens in females, and the evolutionary pathway of the *Gleditsia* reproductive system.

Conflicts of interest

There are no conflicts of interest relating to this paper.

Acknowledgements

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References

Ainsworth C (2000) Boys and girls come out to play: the molecular biology of dioecious plants. *Annals of Botany* **86**, 211–221. doi:10.1006/anbo.2000.1201

Anderson GJ, Symon DE (1989) Functional dioecy and andromonoecy in *Solanum*. *Society for the Study of Evolution* **43**, 204–219.

Barrett SCH (2002) The evolution of plant sexual diversity. *Nature Reviews. Genetics* **3**, 274–284. doi:10.1038/nrg776

Barrett SCH, Hough J (2013) Sexual dimorphism in flowering plants. *Journal of Experimental Botany* **64**, 67–82. doi:10.1093/jxb/ers308

Bruneau A, Klitgaard BB, Prenner G, Fougère-Danezan M, Tucker SC (2014) Floral evolution in the Detarieae (Leguminosae): phylogenetic evidence for labile floral development in an early-diverging legume lineage. *International Journal of Plant Sciences* **175**, 392–417. doi:10.1086/675574

Burkart A, Troncoso de Burkart NS, Bacigalupo NM (1987) 'Flora Ilustrada de Entre Ríos (Argentina). Dicotiledóneas Metaclamídeas: Salicales a Rosales (incluso Leguminosas).' (Colección Científica, Instituto Nacional de Tecnología Agropecuaria: Buenos Aires, Argentina)

Charlesworth D (1984) Androdioecy and the evolution of dioecy. *Biological Journal of the Linnean Society. Linnean Society of London* **22**, 333–348. doi:10.1111/j.1095-8312.1984.tb01683.x

Charlesworth D, Guttman DS (1999) The evolution of dioecy and plant sex chromosome systems. In 'Sex determination in plants'. (Ed. CC Ainsworth) pp. 25–49. (BIOS: Oxford, UK)

Di Rienzo JA, Guzmán AW, Casanoves FA (2002) A multiple-comparisons method based on the distribution of the root node distance of a binary tree. *Journal of Agricultural Biological & Environmental Statistics* **7**, 129–142. doi:10.1198/10857110260141193

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2011) 'InfoStat, versión 2011.' (Grupo InfoStat, FCA, Universidad Nacional de Córdoba: Córdoba, Argentina)

Freeman DC, Lovett Doust J, El-Keblawy A, Miglia KJ, Durant McArthur E (1997) Sexual specialization and inbreeding avoidance in the evolution of dioecy. *Botanical Review* **63**, 65–92.

Gordon D (1966) A revision of the genus *Gleditsia* (Leguminosae). PhD dissertation, Indiana University, Bloomington, IN.

Hoffman MT (1992) Functional dioecy in *Echinocereus coccineus* (Cactaceae): breeding systems, sex ratios, and geographic range of floral dimorphism. *American Journal of Botany* **79**, 1382–1388. doi:10.2307/2445137

Hokche O, Ramirez N (1990) Pollination ecology of seven species of *Bauhinia* L. (Leguminosae: Caesalpinioideae). *Annals of the Missouri Botanical Garden* **77**, 559–572. doi:10.2307/2399520

Kalin Arroyo MT (1981) Breeding systems and pollination biology in Leguminosae. In 'Advances in legume systematic'. (Eds RM Polhill, PH Raven) pp. 733–769. (Royal Botanic Gardens, Kew: London)

Knapp S, Persson V, Blackmore S (1998) Pollen morphology and functional dioecy in *Solanum* (Solanaceae). *Plant Systematics and Evolution* **210**, 113–139. doi:10.1007/BF00984731

Lepart J, Dommée B (1992) Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? *Botanical Journal of the Linnean Society* **108**, 375–387. doi:10.1111/j.1095-8339.1992.tb00252.x

Lewis JP (1981) La vegetación de la Provincia de Santa Fe. *Sociedad Argentina de Estudios Geográficos GAEA* **9**, 121–148.

Lloyd DG (1975) The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica* **45**, 325–339. doi:10.1007/BF01508307

Lloyd DG, Webb CJ (1977) Secondary sex characters in plants. *Botanical Review* **43**, 177–216. doi:10.1007/BF02860717

Mayer SS, Charlesworth D (1991) Cryptic dioecy in flowering plants. *Trends in Ecology & Evolution* **6**, 320–325. doi:10.1016/0169-5347(91)90039-Z

Obeso JR (2002) The costs of reproduction in plants. *New Phytologist* **155**, 321–348. doi:10.1046/j.1469-8137.2002.00477.x

Pensiero JF, Gutiérrez HF, Luchetti AM, Exner E, Kern V, Brnich E, Oakley L, Prado D, Lewis JP (2005) Flora vascular de la provincia de Santa Fe. 'Claves para el reconocimiento de las familias y géneros. Catálogo sistemático de las especies'. (Universidad Nacional del Litoral: Santa Fe, Argentina)

Perduca MJ, Spotti MJ, Santiago LG, Judis MA, Rubiolo AC, Carrara CR (2013) Rheological characterization of the hydrocolloid from *Gleditsia amorphoides* seeds. *Lebensmittel-Wissenschaft + Technologie* **51**, 143–147. doi:10.1016/j.lwt.2012.09.007

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2011) 'nlme: linear and nonlinear mixed effects models. R package version 3.1–131.' Available at https://CRAN.R-project.org/package=nlme [verified 18 December 2017].

Prola G (2011) '*Gleditsia amorphoides* seedless pod extract and its use as an agricultural adjuvant.' US patent application 13/186, 664, 20 July.

R Core Team (2011) 'R: a language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna)

Raven PH, Polhill RM (1981) Biogeography of the Leguminosae. In 'Advances in legume systematic'. (Eds RM Polhill, PH Raven) pp. 27–34. (Royal Botanic Gardens, Kew: London)

Richards AJ (1986) 'Plant breeding systems.' 1st edn. (George Allen and Unwin: London).

Rothman B, Rique T (1959) Las gomas galactomananos y la goma de Espina Corona en la industria alimenticia. *Revista de la Sociedad Química de México* **3**, 379–391.

Schnabel A, Hamrick JL (1990) Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). *American Journal of Botany* **77**, 1060–1069. doi:10.2307/2444577

Schnabel A, Wendel JF (1998) Cladistic biogeography of *Gleditsia* (Leguminosae) based on NDHF and RPL16 chloroplast gene sequences. *American Journal of Botany* **85**, 1753–1765. doi:10.2307/2446510

Strittmatter LI, Negrón-Ortiz V, Hickey RJ (2002) Subdioecy in *Consolea spinosissima* (Cactaceae): breeding system and embryological studies. *American Journal of Botany* **89**, 1373–1387. doi:10.3732/ajb.89.9.1373

Strittmatter LI, Negrón-Ortiz V, Hickey JR (2006) Comparative microsporangium development in male-fertile and male sterile flowers of *Consolea* (Cactaceae): when and how does pollen abortion occur. *Grana* **45**, 81–100. doi:10.1080/00173130500537101

Sun B-L, Zhang Ch-Q, Lowry PP II, Wen J (2009) Cryptic dioecy in *Nyssa yunnanensis* (Nyssaceae), a critically endangered species from tropical eastern Asia. *Annals of the Missouri Botanical Garden* **96**, 672–684. doi:10.3417/2008015

Tortorelli LA (2009) 'Maderas y bosques Argentinos.' (Orientación Gráfica Editora: Buenos Aires)

Missouri Botanical Garden (2017) Tropicos: *Gleditsia inermis* L. Available at http://www.tropicos.org/Name/13000997 [Verified 27 January 2017]

Tucker SC (1987) Floral initiation and development in legumes. In 'Advances in legume systematics'. (Eds CH Stirton) pp. 183–239. (Royal Botanic Gardens, Kew: London)

Tucker SC (1988) Dioecy in *Bauhinia* resulting from organ suppression. *American Journal of Botany* **75**, 1584–1597. doi:10.2307/2444708

Tucker SC (1991) Helical floral organogenesis in *Gleditsia*, a primitive Caesalpinioid legume. *American Journal of Botany* **78**, 1130–1149. doi:10.2307/2444901

Tucker SC (2000) Floral development and homeosis in *Saraca* (Leguminosae: Caesalpinioideae: Detarieae). *International Journal of Plant Sciences* **161**, 537–549. doi:10.1086/314278

Tucker SC (2003) Floral development in legumes. *Plant Physiology* **131**, 911–926. doi:10.1104/pp.102.017459

Ulibarri EA (1997) 32-Fabaceae. In 'Flora fanerogámica Argentina, proflora CONICET'. (Ed. AT Hunziker) pp. 3–26. (Estudio Sigma: Buenos Aires)

Verdú M (2004) Physiological and reproductive differences between hermaphrodites and males in the androdioecious plant *Fraxinus ornus*. *Oikos* **105**, 239–246. doi:10.1111/j.0030-1299.2004.12998.x

Verdú M, Montilla AI, Pannell JR (2004) Paternal effects on functional gender account for cryptic dioecy in a perennial plant. *Proceedings. Biological Sciences* **271**, 2017–2023. doi:10.1098/rspb.2004.2836

Wolf DE, Takebayashi N (2004) Pollen limitation and the evolution of androdioecy from dioecy. *American Naturalist* **163**, 122–137. doi:10.1086/380493

Zarlavsky GE (2014) 'Histología vegetal, técnicas simples y complejas.' (Sociedad Argentina de Botánica: Buenos Aires)

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