RESEARCH PAPER

# Fire frequency effects on cleistogamy expression and progeny performance in *Cologania broussonetii*

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## ABSTRACT

- Increased fire frequency usually erodes microenvironmental conditions, causing a drastic limitation of edaphic resources. Thus, the production of permanently closed-small flowers (cleistogamous, CL) should increase in sites with high fire frequency as this implies a less expensive reproductive assurance strategy. However, because open, insect-pollinated flowers (chasmogamous, CH) have the potential capacity to outcross *via* pollinators, CH progeny produced at any site should outperform selfed CL progeny.

- We evaluate the effect of fire frequency on the relative production of CL/CH flowers and fruits, and their seed set, along with several progeny performance parameters in *Cologania broussonetii* (Fabaceae), a resprouting herb with dimorphic cleistogamy native to the Chaco Serrano.

- Fire frequency increased cleistogamy expression, reaching extreme levels in high fire frequency sites. Seed set was similarly high for both CH and CL flowers in the unburned condition, while in burned sites the few developed CH flowers set more seeds than CL flowers. However, progeny performance was similar between CH and CL progeny at each and across all fire frequency conditions.

- Cleistogamy expression in *C. broussonetii* is maximized in abiotically degraded frequently burned habitats, although the selfed CL progeny is as successful as potentially outcrossed CH progeny. Fire frequency may decreased floral size and abundance, selecting for autogamous reproduction, which restricts not only the genetic potential of plant populations but also the resources offered to pollinators. At the community level, increased cleistogamy expression may potentially have negative implications for non-cleistogamous, more outcrossing species surviving in frequently burned environments.

## INTRODUCTION

Cleistogamy is a dimorphic flowering system that implies the production of both permanently closed small flowers (cleistogamous, CL), which are obligately selfed, and open, insect-pollinated flowers (chasmogamous, CH), potentially capable of outcrossing (Uphof 1938; Maheshwari 1962; Lord 1981; Culley & Klooster 2007; Stojanova *et al.* 2020). Cleistogamy is widespread across angiosperms, present in at least 50 families, evolving multiple times in their evolutionary history, suggesting this system may confer advantages to the fitness of individuals (Culley & Klooster 2007). CL flowers are energetically less expensive to produce in comparison with CH flowers, resulting in higher resource available for seed production. In addition to securing the production of seeds, CL progeny possess maternal genetic information, preserving locally adapted genes (Schoen & Lloyd 1984; Culley & Klooster 2007). CH flowers, in contrast, when cross-pollinated, produce genetically diverse progeny, thereby maintaining or increasing genetic diversity

(Culley & Klooster 2007; Koontz *et al.* 2017). While the production of CL and CH flowers can be determined by genetic factors, biotic and abiotic factors are highly relevant for triggering the occurrence of cleistogamy as either an adaptive strategy or simply as a plastic response to enviromental changes (Clay 1983; Schoen & Lloyd 1984; Sternberger *et al.* 2020).

Environmental factors such as soil moisture and nutrition, light levels and plant size may influence the production of CL and CH flowers (Lu 2002; Munguía-Rosas *et al.* 2013; Furukawa *et al.* 2020). At the microsite scale, abiotic resource limitation usually increases cleistogamy expression, as this phenotype provides a high ratio of fitness/cost returns, maximizing reproductive success (Lloyd 1984; Veena & Nampy 2019; Stojanova *et al.* 2020; Sternberger *et al.* 2020). In addition, biotic factors, such as pollinator activity, can influence cleistogamy expression, as CL flowers can assure seed production *via* selfing when floral visitors are absent or unpredictable (Culley & Klooster 2007; Panique & Caruso 2020). Thus, the frequency and intensity of both biotic and abiotic stress factors can favour

cleistogamy, expressing different reproductive strategies among populations growing in different environments.

Fire is one of the most important disturbances across the Earth that modulates the ecological and evolutionary dynamics of plants and animals in many ecosystems (Bond *et al.* 2005; Pausas & Keeley 2009; Pausas 2019). Currently, anthropic activities have altered natural fire regimes, increasing their frequency or severity and thereby affecting multiple biotic and abiotic variables in the post-fire habitat (Koltz *et al.* 2018; Keeley & Pausas 2019). Several studies show that the increase in fire frequency can trigger high levels of environmental stress, specifically causing a drastic limitation of edaphic resources in many regions (*e.g.* Pellegrini *et al.* 2015, 2018; Carbone & Aguilar 2016, Kowaljow *et al.* 2019; Giorgis *et al.* unpubl.). Although fire is known to promote flowering in many enviroments (*e.g.* North American grasslands and pinelands), recurrent fires may reduce flower size and the abundance of floral resources for pollinators (*e.g.* South American seasonally dry forests), potentially increasing selfing rates, reaching extreme levels in CL species. Further, although fire frequency can negatively affect the floral visitor assemblage, the pollinators have shown many strategies to colonize the post-fire area (Peralta *et al.* 2017; Carbone *et al.* 2019; Lazarina *et al.* 2019). Despite the fact that some studies mention fire as a potential factor that affects cleistogamy frequency (Campbell *et al.* 1983; Gopinathan & Babu 1987; Herndon 1988; Cheplick 1994), there is still no empirical assessment that relates any particular fire regime parameter with cleistogamy expression and even less with the performance of the CL progeny.

Here we evaluate the effect of fire frequency on the floral expression and on progeny performance of *Cologania broussonetii* (Fabaceae), a resprouting herb with dimorphic cleistogamy native to the Chaco Serrano. Specifically, in sites with different fire frequencies, we evaluate the relative production of CL/CH flowers and fruits, and their seed set, as well as seed mass, germination, survival and growth as progeny performance parameters. Because increased fire frequency reduced soil quality, inducing abiotic resource limitation in the studied burned sites (Carbone & Aguilar 2016), we predict that cleistogamy expression will increase in sites with high fire frequency as it implies a less expensive reproductive assurance strategy. Also, because CH flowers have the potential capacity to outcross *via* pollinators, we hypothesize that CH progeny outperform selfed CL progeny, irrespective of the fire frequency.

## MATERIAL AND METHODS

### Study species

*Cologania broussonetii* (Balb.) DC. (Fabaceae) is a perennial herb, widely distributed in subtropical mountain ecosystems from the United States to central Argentina, being the only species of the genus in southern South America (Turner 1992). This species is a common herbaceous vine from the Chaco Serrano ecoregion (Giorgis 2011), especially present in fire-prone environments (Carbone & Aguilar 2016). It has rhizomes and a woody xylopodium, which allow individuals to survive fires and to regenerate by belowground resprouting a few days after burning (*i.e.* obligate resprouters). Individuals set fruits early within the growing season following the fire event (Carbone & Aguilar 2016). The species has low vegetative multiplication

**Key message** Cleistogamy expression is increased in Chaco Serrano habitats with frequent fires, although their selfed progeny is as successful as potentially outcrossed chasmogamous progeny across different fire frequency conditions.

ability; therefore sexual reproduction is the main strategy for long-term population viability.

*Cologania broussonnetii* presents dimorphic cleistogamy, where two flower types are produced in the same individual: chasmogamous (CH, open, insect-pollinated) flowers and cleistogamous (CL, closed, self-fertilized) flowers (Fig. 1). While their typical papilionate CH flowers have the capacity for autonomous self-pollination, animal pollination significantly increases fruit and seed production (Musicante & Galetto 2008). Thus, autonomous self-pollination confers reproductive assurance to CH flowers but outcrossing improves the quantity and quality of fruits and seeds (Musicante & Galetto 2008). Chasmogamous flowers are mostly pollinated by bumblebees (*Bombus* spp.), which visit their flowers searching for nectar at rather low frequencies (Musicante & Galetto 2008; Carbone 2017). Surprisingly, the only mention of development of CL flowers in *C. broussonnetii* has gone mostly unnoticed (McVaugh 1987). Cleistogamous flowers show reduced size and complexity compared to CH flowers (Fig. 1), and are commonly located in stem nodes above or below CH flowers, or even in the same axillary inflorescences (McVaugh 1987). In addition, CL fruits are similar to CH fruits in morphology but are smaller, present six to nine seeds, and at their maturity CL fruits have remnants of the small calyx and the visible reflex style (McVaugh 1987; Turner 1992), which allow their differentiation in the field.

### Studied sites and sampling

The field sampling was conducted in the eastern hillsides of Sierras Chicas from Córdoba, Argentina. The vegetation consists of subtropical dry forest intermingled with shrublands and grasslands, consisting of a composite mosaic of physiognomies, which can vary in plant composition. This complex landscape configuration is determined by the impact of human disturbances such as fire and livestock grazing pressures. Over the
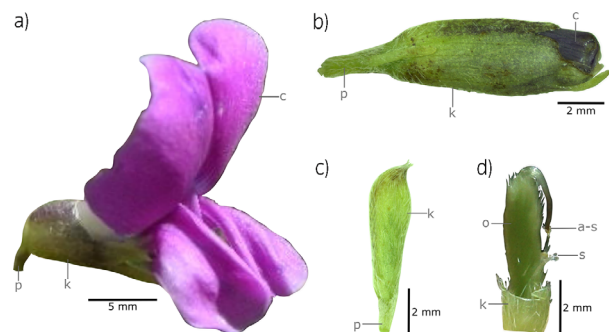


**Fig 1.** Flower morphology in *Cologania broussonnetii:* chasmogamous flower in anthesis (a), chasmogamous flower bud (b), cleistogamous flower (c), and dissected cleistogamous flower (d). Letters indicate: *p*, peduncle; *k*, calyx; *c*, corolla; *s*, stamens; *o*, ovary; *a-s*, anther-stigma union.

past decades, fire regime has changed in Sierras Chicas due to human activities linked to urbanization and cattle production, increasing the frequency of fires in shrublands and grasslands at the end of the dry season (Miglietta 1994; Argañaraz 2016). Currently, the Sierras Chicas is the mountain system of central Argentina most affected by fire in total area and frequency, with 297.125 ha out of 812.663 ha burned between 1999 and 2019, which is equivalent to 36.6% of its area, and with sites that register up to five or six fires in just 17 years (Argañaraz *et al.* 2015). We compiled the fire history of the past 24 years (1991–2015 period) from different databases and covering approximately 40 km$^2$ (31°05′38.53″ S to 31°09′11.73″ S and 64°24′10.49″ W to 64°20′40.35″ W). We used field fire records (Giorgis *et al.* 2013) and Landsat TN and ETM satellite images (Argañaraz *et al.* 2015). We selected nine sites with different fire regimes: three *unburned* sites, three *low fire frequency* sites with one to two fires, and three *high fire frequency* sites with three to four fire events; see Carbone & Aguilar (2016) for spatial distribution and specification of studied sites. All the burned sites shared the same time elapsed since the last fire (which occurred in 2011; *i.e.* three post-fire years). All sites were selected with the criteria of comparable altitudinal position (820–1200 m a.s.l.), slope exposure (N) and topographic position (middle slope). Sampled sites are located in private properties with similarly low stocking rates and separated by a minimum distance of 500 m from each other. The unburned sites were represented by a physiognomy of open native forest, while burned sites had a shrubland structure (Carbone *et al.* 2017). In a previous contribution, we found significantly decreased soil quality in the burned sites where this study was conducted (Carbone & Aguilar 2016).

In each of the nine sites, we marked 12 adult individuals of *C. broussonetii* at their vegetative stage in the 2014–2015 warm season, 3 years after the last fire (2011) for most of the burned sites. All sampled individuals were relatively similar in size, and thereby of approximately similar age. Individuals were separated from each other by a minimal distance of 3 m. We collected 20 CH and CL flowers in the sites where both flower types were produced simultaneously in the same plant, which were fixed in 70% alcohol. These flowers were subjected to detailed morphological measurements in a stereoscopic microscope to determine their differences.

To quantify cleistogamy expression, we registered weekly the number of CH and CL flowers and fruits produced per plant across all sites from December 2014 to March 2015. These measurements were conducted on the same individuals throughout the flowering period, except for one or two individuals per site that died and were replaced by other adult plants of the population. All CH and CL fruits produced by each individual were collected at maturity, counting both the number of seeds and unfertilized ovules per fruit. We calculated seed set as the number of healthy seeds per fruit/ number of ovules per flower.

To evaluate offspring performance, we selected 80 seeds from each of six maternal plants per site that had produced CH and CL progeny to carry out the experiments (N = 40 CH and 40 CL seeds per maternal plant). Progeny performance at multiple life stages was estimated as seed mass, germination, survival and growth rate of seedlings in controlled conditions. Seed mass was individually weighed with a precision digital scale in three randomly selected healthy CH and CL seeds per each maternal plant from each site across fire frequency conditions. Germination levels were quantified in 20 healthy CH and CL seeds from six maternal plants per site in June 2015. Seeds were individually scarified with sandpaper, and placed on filter paper in Petri dishes, which were carefully tabulated by the maternal and flower type origin of each seed. Seeds were moistened with distilled water, placed in a germination chamber at 25 °C and 12-h photoperiod, and monitored daily to quantify the proportion of germinated seeds. Three germinated seeds per maternal plant (9 sites, 6 maternal plant per site, 3 CH and CL seedlings per maternal plant, totalling 324 seedlings) were then sown in tubular nylon pots (identified by site, CH/CL origin, and maternal plant) of 12-cm diameter and 20-cm depth filled with a mixture of 40% sand and 60% blended commercial soil, which were used for all seedling in a greenhouse with controlled conditions. We registered dead seedlings weekly to calculate survival. We placed a wooden rod in each pot to support and facilitate growth of these climbing plants. Pots were grouped in trays (40–50 per tray) that were randomly interchanged around the greenhouse every 2 weeks. Pots were manually watered twice a week. We monitored the seedling growth every 15 days throughout 3 months (*i.e.* 96 days after transplant) and measured the total height from cotyledon node to apical meristem of each individual seedling. We calculated the relative growth rate in height (RGR, cm/day$^{-1}$; Hunt 1990) per seedling as: $(LnH_2 - LnH_1 / T_2 - T_1)$, where $LnH_1$ and $LnH_2$ are the natural logarithm of seedling height measured every 15 days across 3 months (*i.e.* $T_2 - T_1 = 15$). Finally, we estimated a multiplicative performance function across all measured life stages: reproductive success, germination, seedling survival and seedling growth. For each individual plant, this cumulative performance was calculated by multiplying performance differentiating by flower type (CH/CL) as: seed set, percentage seed germination, percentage seedling survival and relative growth rate of seedlings at the end of the experiment.

## Data analysis

We performed different linear mixed models (LMM) and generalized linear mixed models (GLMM) extensions according to the error distribution of each response variable. We used GLMM to test the effect of fire frequency on the proportion of CL flowers and fruits, which were calculated based on the number of CL and CH flowers and fruits with a binomial family distribution (*glmer* function from *lme4* package). In these analyses, fire frequency condition was the fixed effect with three levels (unburned, low fire frequency and high fire frequency), and site was used as a random factor with nine levels nested within fire frequency. To evaluate the assumption of homogeneity of variances, we estimated overdispersion by calculating the parameter of scale, $\hat{c} = \Sigma(\text{Pearson residuals}^2)/\text{df}$. When overdispersion was observed in the explanatory models ($\hat{c} > 1.2$), this was corrected using selection of models by the Akaike information criterion for overdispersed data, correcting also for small sample size (QAICc, function *dredge* of the *MuMin* package). The significance of the fixed factor was determined by the Wald $Z$ statistic, the $P$-value and confidence intervals based on averaging the multiple models according to their relative weight (*model.avg* function of the *MuMin* package). This method (conditional GLMM) considers the variability attributed to both fixed and random factors.

To assess whether seed set (seed/ovule ratio) differed among fire frequency conditions and between CH/CL fruits (fixed effects), we used a GLMM model with mature seeds and undeveloped seeds (ovules – mature seeds) per fruit with binomial family. Fruit, maternal plant and site were used as random effects. Significance of the fixed factors and their interaction in this model was performed with analysis of deviance of nested models. To evaluate seed mass differences among fire frequency and CH/CL origin we performed LMM with the same fixed effect structure previously mentioned. To test the effect of fire frequency condition and CH/CL origin on seed germination, we used number of germinated seed and number of non-germinated seed as response variable in a GLMM binomial model with the same fixed and random effects structure.

To assess whether seedling survival differed among fire frequency and CH/CL origin, we analysed the probability of surviving and dead seedlings over the 96 days of the experiment with Cox proportional-hazards models, which examine the relationship between survival distribution of the seedlings and one or more predictors. For this we used LMM with fire frequency and CH/CL origin as fixed effects, and site and maternal plant as random factors, applying the *coxme* function from the *coxme* package. Significance of the fixed (and their interaction) and random factors in this model was performed with analysis of deviance of nested models.

We analysed the relative growth rate in height of seedlings among different fire scenarios and CH/CL origin with LMM, and applied the effect of temporal pseudoreplication involving repeated measurements on the same seedlings over time. For this, an autoregressive correlation structure was adjusted to model the dependency between measurement intervals (function *corAR1* of the *nlme* package), which are temporally correlated with each other and this correlation decreases as the temporal separation increases. Finally, the net effect on fire frequency condition and flower type on the cumulative performance was assessed with LMM. Significance of the fixed factors and their interaction was tested with likelihood-ratio test of nested models. All analyses were performed in R version 3.6.1 (R Core Team 2020), and the databases are available in the Supplementary Material. Datasets used in the analyses are found in Table S2 and Table S3.

# RESULTS

## Flower and fruit morphology

Cleistogamous flowers were represented by the fused calyx at their apex, where the fertilization occurs spontaneously. CL flowers were significantly smaller in peduncle, calyx, stamen number and ovary size than CH flowers (Table 1, Fig. 1). CL flowers had no corolla nor nectary, and their anthers and stigma were in close contact (Fig. 1). The ovule number in CL flowers was about half that found in CH flowers. CL fruits were significantly shorter than CH fruits (Table 1). Both floral types were produced in the same individual plant, and even within the same axillary inflorescence. These morphological traits were unchanged across all fire frequency scenarios. Although CL flowers developed earlier, there was not phenological differentiation in the production of the two floral types.

## Cleistogamy expression and reproductive performance

The proportion of CL flowers was significantly higher in high fire frequency condition ($\bar{X}$ = 0.97, SD = 0.09) in comparison to low fire frequency ($\bar{X}$ = 0.81, SD = 0.20) and to unburned conditions ($\bar{X}$ = 0.42, SD = 0.23). Despite the proportion of CL flowers in the low fire frequency being twice that in unburned sites, there were no statistically significant differences between them, which is likely due to limited statistical power (Fig. 2a, Table S1). CL fruits followed this same pattern of increase with fire frequency, with most of the fruits (98.3%) produced by CL flowers from plants growing in high fire frequency sites (Fig. 2b, Table S1). Seed set of CH and CL flowers responded differently according to fire frequency conditions ($\chi^2$ = 79.398, $P$ < 0.0001). In the unburned condition, seed set was similarly high for both CH and CL flowers, while in both low and high fire frequency conditions the few developed CH flowers set more seeds than CL flowers (Fig. 3a). In addition, CL flowers set fewer seeds in both burned conditions than in the unburned sites ($Z_{\text{unburned-low}}$ = 3.921, $P$ < 0.0001; $Z_{\text{unburned-high}}$ = 2.429, $P$ = 0.015; Fig. 3a). In contrast, CH flowers set a similar number of seeds amongst them and across burned and unburned sites.

**Table 1.** Morphological differentiation of chasmogamous (CH) and cleistogamous (CL) flowers and fruits in *Cologania broussonetii* in the Chaco Serrano. Values in the same row followed by different letters are significantly different at 0.05 level according to general and generalized linear models.

| | CH | | | CL | | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | n | Mean | SD | n | Statistic* |
| Peduncle length (mm) | 8.78[a] | 1.41 | 80 | 2.91[b] | 0.73 | 80 | $E$ = −5.87, $t$ = −33.04, $P$ < 0.0001 |
| Calyx length (mm) | 12.4[a] | 1.38 | 20 | 6.08[b] | 0.67 | 20 | $E$ = −6.28, $t$ = −18.29, $P$ < 0.0001 |
| Corolla | Present | | | Absent | | | |
| Anther number | 10[a] | 0 | 20 | 2[b] | 0.39 | 20 | $E$ = −1.63, $Z$ = −9.34, $P$ < 0.0001 |
| Nectary | Present | | | Absent | | | |
| Ovary length (mm) | 8.25[a] | 0.51 | 20 | 5.15[b] | 0.61 | 20 | $E$ = −3.11, $t$ = −17.40, $P$ < 0.0001 |
| Ovule number | 12.52[a] | 1.43 | 348 | 6.74[b] | 1.22 | 1134 | $E$ = −0.60, $Z$ = −28.45, $P$ < 0.0001 |
| Fruit length (cm) | 4.92[a] | 0.48 | 348 | 3.16[b] | 0.48 | 1134 | $E$ = −1.76, $t$ = −60.31, $P$ < 0.0001 |

*$E$ (Estimate) indicates the magnitude of the difference between floral types (CH *versus* CL).
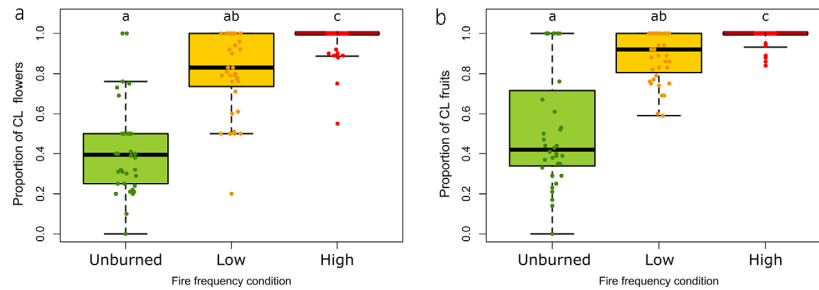
**Fig 2.** Proportion of cleistogamous (CL) flowers (a) and fruits (b) produced by individuals from *Cologania broussonetii* in unburned, low and high fire frequency sites from Chaco Serrano. The cleistogamy proportion is calculated as the ratio between the number of cleistogamous flowers and the number of total flowers (cleistogamous + chasmogamous) in 12 individual plants per site throughout the 2014–2015 growing season. The same calculation is applied to the proportion of cleistogamous fruits. Box lines represent the upper and lower extremes (whiskers), the third and first quartiles (hinge) and the median. Graph dots represent the observed cleistogamy level of each individual plant. Different letters indicate statistical significant differences ($P < 0.05$) in parameter value between fire frequency conditions, according to GLMM analyses. The statistical tests are shown in Table S1.
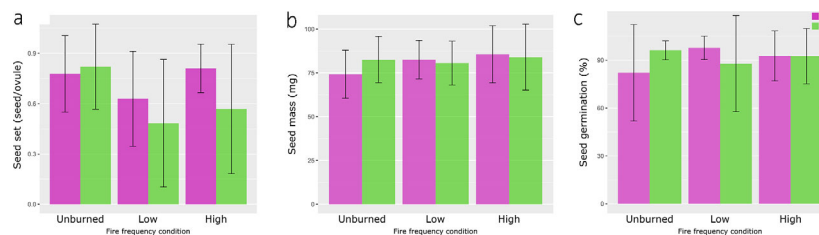


**Fig 3.** Seed set (a), seed mass (b) and seed germination (c) in *Cologania broussonetii* of chasmogamous (CH, purple) and cleistogamous (CL, green) flowers in unburned, low fire frequency (1 or 2 fires) and high fire frequency sites (3 to 4 fires) in the 1991–2015 period. Values are mean ± SD. The LMM indicate significant interactions between seed type (CH/CL) and fire frequency condition for seed set ($\chi^2 = 79.398$, $P < 0.0001$, $N_{total} = 937$ fruits) and for seed mass ($\chi^2 = 12.024$, $P = 0.0025$, $N_{total} = 302$).

## Progeny performance

The mass of CL and CH seeds was different depending on the fire frequency condition ($\chi^2 = 12.024$, $P = 0.0025$). While CL seed mass was similar across all fire frequency conditions, CH seeds presented lower mass ($-9.8$ mg on average) in unburned sites compared to both low and high fire frequency conditions (Fig. 3b). Germination levels were similarly high between both CH ($90.6 \pm 21.1\%$) and CL ($92.1 \pm 20.5\%$) seeds and also among sites with different fire frequency (Fig. 3c). Seedling survival after 85 days post-transplant was relatively high and was not affected by different floral types (CH *versus* CL; $\chi^2 = 1.538$, $P = 0.215$) nor by fire frequency conditions ($\chi^2 = 1.6456$, $P = 0.439$; Fig. 4). Relative growth rate was similar between CH and CL progeny (likelihood ratio $= 0.049$, $P = 0.825$) at each and across all fire frequency conditions (likelihood ratio $= 3.130$, $P = 0.209$), without interactions between these factors (likelihood ratio $= 0.119$, $P = 0.942$; Fig. 5). Finally, the cumulative performance across different life stages was not affected by fire frequency and flower type (and their interaction, Figure S1). Therefore, CL progeny is as successful in terms of net individual fitness as CH progeny, regardless of the fire frequency condition.

## DISCUSSION

Dimorphic cleistogamy is thought to be an advantageous reproductive strategy that allows for plastic responses in sexual reproduction depending on the quality of pollination and abiotic resource environment (*e.g.* Ansaldi *et al.* 2019). In this study we found that cleistogamy expression in *C. broussonetii* is maximized in abiotically degraded frequently burned habitats from Chaco Serrano that apparently did not show depleted pollination fauna. The performance of selfed CL progeny was as successful as potentially outcrossed CH progeny. Our results imply that increased fire frequency may decrease floral size and abundance by selecting autogamous reproduction. Not many studies have evaluated the effects of fire on cleistogamy expression. In fact, the only study that directly related fire effects with cleistogamy is reported for an endemic legume (*Clitoria fragrans*) from fire-maintained scrub and sandhill communities in central Florida, which mainly reproduced by CL flowers in the first post-fire years (Lewis 2000). To our knowledge, the present study is the first evaluating the effect of fire frequency on the intraspecific variability of cleistogamy and the performance of its progeny.

In frequently burned conditions, abiotic resource limitations, especially decreased soil nutrients and water availability, are the main drivers of change in many plant traits such as specific leaf area, leaf dry matter content and plant nutrients (*e.g.* Keeley *et al.* 2011; Dantas *et al.* 2013; Carbone & Aguilar 2016). Decreased soil quality has been assiduously reported in burned Chaco Serrano environments (Kowaljow *et al.* 2019; Giorgis *et al.* unpubl.), and particularly for the study sites where this study was conducted (Carbone & Aguilar 2016, 2017). Thus, it is not unexpected that species with dimorphic cleistogamy from the Chaco Serrano, such as *C. broussonetii*, are able to express almost full cleistogamy in stressed and
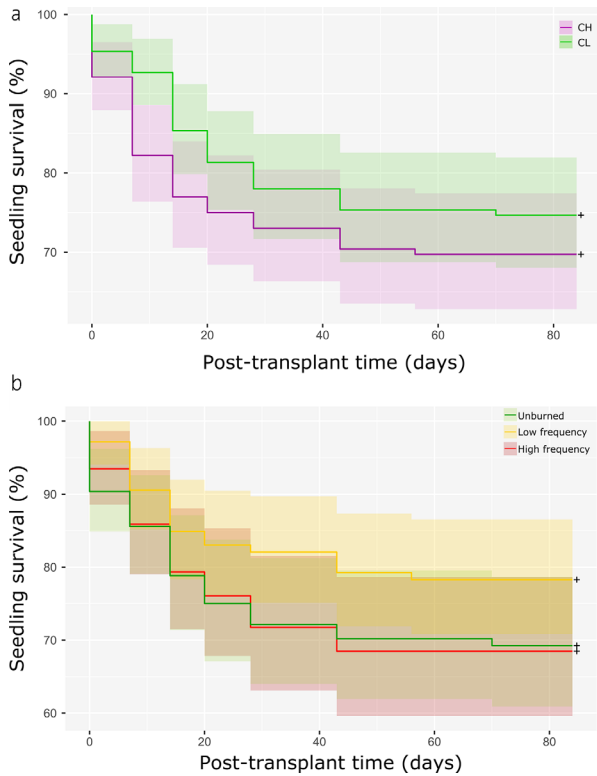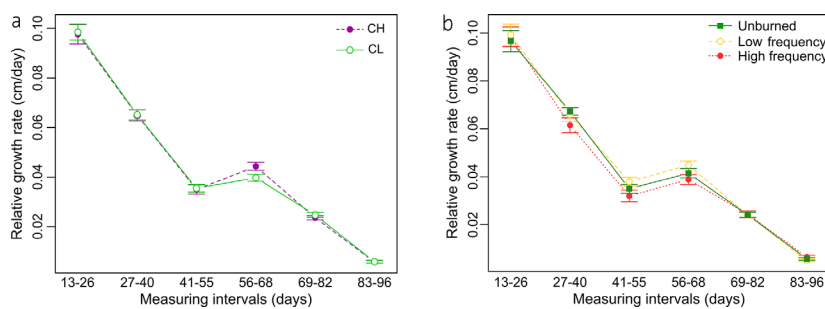
**Fig 4.** Seedling survival of *Cologania broussonetii* up to 84 days after transplant in the greenhouse of different progeny (a): chasmogamous (CH) and cleistogamous (CL); and from different fire frequency conditions (b): unburned, low fire frequency (1 or 2 fires) and high fire frequency sites (3–4 fires). Shaded bands around each line denote 95% confidence intervals.

depleted environments, triggered by increased fire frequency, as CL flowers cost one third as much as CH flowers (Culley & Klooster 2007). Moreover, such decreased soil fertility has probably determined the lower seed set of CL flowers in burned sites compared to the seed- et of CL flowers from unburned conditions, as has been previously similarly observed for the seed set of two other resprouting native *Fabaceae* in these same study sites (Carbone & Aguilar 2017).

The production of cleistogamous flowers has been shown to increase under environmental conditions that may also reduce pollinator abundance (*e.g.* Oakley *et al.* 2007; Panique &

Caruso 2020). Interestingly, pollinator richness, abundance and composition of two sympatric legumes were similar across the studied burned and unburned sites (Carbone & Aguilar 2017). One of these species (*Desmodium uncinatum*), is also pollinated by the same bumblebee species as *C. broussonetii* (Musicante & Galetto 2008; Carbone & Aguilar 2017). While we did not evaluate pollination interaction in *C. broussonetii*, we might assume that its pollinators were similarly present across all sites as we observed for *D. uncinatum*. Thus, cleistogamy expression in *C. broussonetii* would be mainly prompted by abiotic factors such as degraded microenvironmental conditions, as documented elsewhere for other CL species (*e.g.* Uphof 1938; Stojanova *et al.* 2020; Furukawa *et al.* 2020; Sternberger *et al.* 2020). However, despite the lack of influence of the pollination environment in the expression of cleistogamy in our study sites, we did observe differences in the relative reproductive success between CH and CL flowers in burned sites, where CH flowers set more seeds than CL flowers in both low and high fire frequency conditions. One initial possibility for such response patterns is that only the most vigorous plants produced CH flowers in low and high fire frequency sites; thus regardless of pollinator visitation, their higher vigour directly contributed to greater seed set. Another possible explanation, however, is that the few CH flowers available in burned sites were visited by pollinators, and hence possibly received higher pollen loads, setting as many seeds as CH flowers of plants from unburned sites. In contrast, seed set was similar between CH and CL flowers in unburned sites, which may indicate that the pollinator effect on CH flowers in these more diverse communities may have been diluted among the higher number of CH flowers offered by *C. broussonetii* and also by the increased flower offer by other species, thereby increasing interspecific competition for pollinators (*e.g.* van der Kooi *et al.* 2016). These aspects of increased floral offer and competition in unburned sites may have also been responsible for the lower seed mass of CH flowers.

In contrast to our initial hypothesis, CH progeny did not outperform CL progeny, but both were similarly fit, irrespective of fire frequency. In other words, CL and CH progeny showed similar performance regardless of the degree of cleistogamy expression of maternal plants and of the fire frequency condition where they grow. While our study has not evaluated selfing rates, the effect of flower type (CH *versus* CL) and pollen source (xenogamous *versus* autogamous) may not be very relevant, particularly in *C. broussonetii*, as the spontaneous self-



**Fig 5.** Relative growth rate in height of *Cologania broussonetii* seedlings growing in controlled greenhouse conditions for 3 months of different progeny (a): chasmogamous (CH, filled circles) and cleistogamous (CL, empty circles); and from different fire frequency sites (b): unburned (squares), low (empty circles) and high fire frequency conditions (filled circles). Values are mean ± SE.

deposition of pollen occurs also in CH flowers (Musicante & Galetto 2008; Carbone 2017). Thus, such potential similar levels of selfing in both CH and CL flowers of *C. broussonetii* across all studied sites would explain the results of not finding differences in any of the progeny performance parameters evaluated at different stages. However, a potential caveat of measuring progeny performance in greenhouse experiments is that it may mask mild or moderate inbreeding depression effects, due to the optimal growth conditions. Thus, we cannot ensure whether inbreeding depression is taking place or not. Further studies on the species, obtaining outcross and selfed progeny from CH flowers through manual pollination treatments and comparing their performance with CL progeny would help better elucidate the relative effects of selfing and outcrossing and the current mating patterns of *C. broussonetii*.

Decreased soil nutrients and water availability in frequently burned sites of Chaco Serrano represents an important abiotic factor on the floral expression of *C. broussonetii*. However, we cannot ascertain whether increased cleistogamy expression is actually adaptive or simply a plastic response to these abiotic factors. Non-pollinator, abiotic agent-mediated changes on floral traits have been observed for other cleistogamous and non-cleistogamous species growing in stressed environments, typically reducing flower size and flower abundance (*e.g.* Culley & Klooster 2007, Strauss & Whittall 2006). Thus, under the current frequency of fires in Sierras Chicas, such decreased edaphic conditions may trigger changes in floral expression on other cleistogamous and non-cleistogamous plant species of the Chaco Serrano community. In fact, there are many herb and shrub species in these communities that show different types and degrees of cleistogamy, such as *Clitoria cordobensis*, *Janusia guaranitica*, *Malvastrum coromandelianum*, *Pavonia aurigloba*, *Sida spinosa*, *Triodanis perfoliata* var. *biflora* and *Wahlenbergia linarioides* (Giorgis 2011; Carbone *et al.* 2017; Kowaljow *et al.* 2019), which may also increase their degree of cleistogamy expression in frequently burned sites. In addition, if reductions in flower size and flower abundance also take place in non-cleistogamous species in these burned habitats, they may shift into predominantly cleistogamy-biased and autogamous plant communities. Such changes would reduce nectar and pollen resources for floral visitors, decreasing the attractiveness to pollinators and thus their abundance, creating a negative feedback for the maintenance of pollinator fauna and potentially decreasing outcrossing rates in animal-pollinated plants (Holtsford & Ellstrand 1992; Strauss & Whittall 2006). Such a situation may have cascading effects for the long-term population viability of mostly outcrossing, non-cleistogamous species.

Future research will endeavour to focus on assessing fire frequency effects on cleistogamy expression and on flower trait variations in non-cleistogamous species at the community level. These important research questions have not yet been posed and will help to understand floral trait evolution and sexual expression in fire-degraded habitats.

## AUTHOR CONTRIBUTIONS

LMC and RA contributed to the study conception and design. Field sampling, material preparation, greenhouse work and data collection were performed by LMC and GC. Statistical analyses and the first draft involved LMC. LMC and RA reviewed, edited and approved the final version of this manuscript.

## CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Cumulative performance of CH and CL progeny in different fire frequency conditions.
**Table S1.** Statistical tests of contrasts between fire frequency categories for proportion of CL flowers and fruits.
**Table S2.** Database of CL proportion.
**Table S3.** Database of seed set, germination, seedling survival, relative growth rate and cumulative performance at individual level.

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