

## RESEARCH PAPER

# Sex-specific reproductive components and pollination ecology in the subdioecious shrub *Fuchsia microphylla*

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## Keywords

Apomixis; breeding system evolution; fruiting male; gynodioecy; pollination ecology; subdioecy.

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## ABSTRACT

**In subdioecious populations, functional female, male and hermaphrodite individuals coexist. Subdioecy may be a transitional state towards dioecy or a breakdown of dioecy, although lability in sex expression may maintain subdioecy as a stable condition. To better understand the ecological aspects involved in sex ratio dynamics and breeding system evolution, we studied the pollination and female fitness components of female and hermaphrodite individuals of the subdioecious shrub *Fuchsia microphylla*. In two natural populations at the Trans-Mexican Volcanic Belt we estimated female frequency and several reproductive components of female and hermaphrodite plants under natural pollination and experimental pollination treatments. Average female frequency was 42%, and on average, 42.5% of hermaphrodites produced fruits. Female plants showed a 17-fold female fertility advantage over hermaphrodites through increased fruit production, as the number of seeds and germination rates did not differ between morphs. Hermaphrodite flowers were larger, with similar nectar production and concentration to female flowers, and pollinators did not show consistent morph preferences. Some hermaphrodites produced fruits autonomously, and female flowers excluded from pollinators produced fruits putatively by apomixis. Fruit production in hermaphrodites, but not in females, was related to height, suggesting increased investment of hermaphrodites in the female function at higher resource status. For sex ratios to be at equilibrium, the female fertility advantage should be reduced about eightfold. However, it may be that hermaphrodites are maintained by producing fruits at no cost to the male function at higher resource status, as the gender plasticity hypothesis proposes.**

## INTRODUCTION

Subdioecy is a breeding system in which individuals that function as females, males and hermaphrodites at lower frequency coexist in a plant population. This is a rare condition, with 32 species reported in 21 families (Ehlers & Bataillon 2007). Subdioecy can be interpreted as a transitional state in the evolution to dioecy (female and male individuals in a population) or as a breakdown of dioecy, although it has been suggested that subdioecy could also be a stable condition (Delph & Wolf 2005; Ehlers & Bataillon 2007). Lability of sex expression, or gender plasticity, may be involved in breeding system evolution and prevent the evolution to complete dioecy (Barrett *et al.* 1999; Delph & Wolf 2005; Ehlers & Bataillon 2007). In the gynodioecious pathway to dioecy, theoretically, the spread and maintenance of females in a population requires a seed fitness advantage relative to hermaphrodites (Lewis 1941; Lloyd 1975, 1976; Charlesworth & Charlesworth 1978), and hermaphrodites are expected to experience frequency-dependent selection for enhanced male function as females spread (Charlesworth 1989). Nevertheless, if adaptive, some individuals may retain their fruiting ability and occasionally produce some fruits. In most subdioecious species there are hermaphrodites labile in

their sex expression and can vary their allocation to female function (Ehlers & Bataillon 2007).

The resource environment may influence gender expression. Delph (1990) hypothesised that labile hermaphrodites may allocate excess resources to the female function in resource-rich environments with no trade-off in pollen production, and function as males under harsh resource conditions. An increased allocation to the female function relative to the male function at larger plant size or more resources may be explained because the resource costs of producing seeds are higher than those of producing pollen, and because male fitness gains are expected to decelerate with increased plant size (or resources), but not female fitness gains (Klinkhamer *et al.* 1997; Ashman 2006). Resource- or size-dependent gender plasticity has been reported in several subdioecious species, in which larger hermaphrodites and hermaphrodites in higher resource environments produce more fruits (Delph & Wolf 2005). If hermaphrodites are more plastic in their allocation to the female function than females, then the seed fertility advantage and sex ratio will vary depending on the resource environment (Delph 2003).

On the other hand, subdioecy is associated with animal pollination, more commonly in the gynodioecious pathway

(Ehlers & Bataillon 2007), which suggests that the interaction with pollinators may play a role in the evolution of subdioecy. In sexually dimorphic, animal-pollinated species, offspring production of unisexuals depends on the interaction with pollinators, and pollinators can affect breeding system evolution by influencing sex ratios through a differential effect on the fertility of the morphs. Pollinator limitation can reduce the difference in female fitness between pure females and hermaphrodites if hermaphrodites are capable of autonomous selfing and do not suffer strong inbreeding depression (Maurice & Fleming 1995; Ehlers & Bataillon 2007). The model of Ehlers & Bataillon (2007) showed that this scenario increases the range of fertility differences (considered under optimal pollination) between hermaphrodites and females that allows labile hermaphrodites to stably persist in a population. A reproductive assurance mechanism in females that could counteract the benefits of autonomous selfing in hermaphrodites is apomixis, the asexual production of seeds, although its occurrence has been poorly explored in dimorphic systems (Richards 2003).

Pollinator preferences can also affect the relative fitness of the morphs, especially under pollinator limitation. In the great majority of gynodioecious species and the few studied cases of subdioecious species, female flowers are smaller and do not produce pollen (Shykoff *et al.* 2003; Cuevas & Lopez 2011), which may result in lower pollinator visitation for this morph (*e.g.* Ashman & Stanton 1991; Delph & Lively 1992; Vaughton & Ramsey 1998; Ashman *et al.* 2000). Moreover, lower nectar production by female flowers can further reduce visitation rates and seed production of female plants (Delph & Lively 1992; Ashman *et al.* 2000).

The genus *Fuchsia* (Onagraceae) contains ~110 species and is animal-pollinated, mostly by hummingbirds. Most species are hermaphroditic, although nine dimorphic species have been reported (Berry *et al.* 2004). *Fuchsia microphylla* belongs to the section *Encliandra*, characterised by the presence of male sterility (Berry *et al.* 2004), and is one of the few subdioecious species of the genus, making it a good system to study breeding system evolution. Here, we evaluate and compare female fitness components of females and hermaphrodites, and consider the pollination and plant resource status contexts under which the reproduction of *F. microphylla* occurs. In particular, we address the following questions: (i) are there differences between female and hermaphrodite individuals in fruit and seed production and offspring performance; (ii) is there evidence of pollen limitation, apomixis in female plants and autonomous fruit production in hermaphrodite plants; and (iii) is plant size correlated to flower and/or fruit production in hermaphrodite and female plants? Individual plant size is expected to reflect the size of the resource pool available for reproduction (Venable 1992), as absolute reproductive investment is closely linked to plant size (Harper & White 1974; Samson & Werk 1986). Because hermaphrodites produce perfect flowers, an increased investment to the female function with higher resource status cannot be detected at this level. We expect fruit production to increase more than flower production with increasing plant size in hermaphrodites, and fruit production to increase more in hermaphrodites than in females if there is sex differential gender plasticity. Finally, (iv) are flower size, floral display and nectar production and concentration similar between morphs, and do pollinators visit one morph more than the other?

## MATERIAL AND METHODS

### Study species and sites

*Fuchsia microphylla* is a relatively common shrub native to the understorey of cool temperate, pine–oak forests of the Trans-Mexican Volcanic Belt, occurring between 2500–3500 m a.s.l. (Rzedowski & Rzedowski 2001). In general, population sizes range between 100–500 individuals (Breedlove 1969); this understorey species occurs mainly in conserved forests (E. Cuevas, personal observation). *F. microphylla* was first described as a gynodioecious species based on morphological characteristics (Breedlove 1969) and later, given that around 90% of hermaphrodites function as males, was defined as a subdioecious species (Arroyo & Raven 1975). The flowers of *F. microphylla* have four sepals and four small purplish-red petals, forming a cylindrical floral tube. Based on evidence of segregating progeny obtained from control hand-pollinations, sex determination is probably under nuclear factors (Arroyo & Raven 1975). There are no apparent morphological differences between flowers of hermaphrodites that do and do not produce fruit. Hermaphrodite flowers have eight stamens and four stigmatic lobes (Arroyo & Raven 1975). Female flowers may be distinguished from hermaphrodite flowers by their shorter floral tube and the presence of vestigial stamens that do not produce pollen (Arroyo & Raven 1975). The flowering season runs from August to January, peaking in late October–early December (E. Cuevas, unpublished data). Bumblebees and hummingbirds have been reported as pollinators (Breedlove 1969; Arizmendi *et al.* 1996). On average female flowers produce between six and 36 seeds (Breedlove 1969).

This study was carried out during 2008 and 2009 in two populations of conserved forest with similar environmental conditions on the Trans-Mexican Volcanic Belt in the state of Michoacán. The population at Cerro Garnica (19°40' N, 100°49' W, 2950 m a.s.l.) is located in a protected natural area of 968 ha. The population at San Jose is on a 5-ha private property (19°41' N, 100°49' W, 2873 m a.s.l.) located within a ~100-ha area of primarily conserved forest. Plant height is similar between populations ( $F_{1,240} = 0.232$ ,  $P = 0.631$ ). The populations are about 2 km apart. The climate is temperate sub-humid (mean annual temperature 12–14 °C and annual rainfall 1200–1400 mm; Romero 1991). The vegetation is pine–oak forest, with *Quercus laurina*, *Q. crassipes*, *Pinus pseudostrobus*, *P. teocote* and *Abies religiosa* as dominant species (Romero 1991).

### Sex ratio and reproductive parameters

Sex ratio was estimated in each population along a 300-m line transect. At Cerro Garnica, a single transect was laid across the population. At San Jose, three parallel transects 100-m long were placed in the central area of the population, separated by 15 m from each other. In late November, the middle of the flowering season, the morphological sex of all flowering plants within transects (Garnica: 107 individuals, San Jose: 153 individuals) was determined as female or hermaphrodite based on three or more open flowers per plant. Chi-square goodness of fit tests were used to test whether female to hermaphrodite sex ratios differ from equality. At the end of the flowering season, we estimated the proportion of hermaphrodites that set at least one fruit (henceforth, fruiting hermaphrodites).

We compared reproductive components of hermaphrodite and female plants. The number of open flowers per plant per sampling day (floral display) was recorded on plants randomly chosen on each of six occasions, spread throughout the flowering season, from September 2008 to January 2009. To compare flower size between morphs, the corolla diameter and length of three open flowers was measured using digital calipers. Fruit production (number of fruits per plant) was recorded twice on the same plant during the flowering season, in October (developing fruits) and January (ripening fruits). All these reproductive components were estimated in at least 12 plants per morph (range: 12–46). Depending on fruit availability, between one and three ripe fruits were collected from eight to nine plants per morph to obtain fresh fruit mass and to estimate the number of viable and aborted seeds per fruit. Aborted seeds can be easily distinguished from potentially viable, developed seeds by their smaller size. The height of hermaphrodite and female plants, for which flower or fruit production was obtained, was recorded to investigate the relation between plant size and flower and fruit production (Barrett *et al.* 1999). Plant height in this species is positively correlated to mean plant width estimated from two measurements in different directions ( $r = 0.347$ ,  $P = 0.033$ ,  $n = 38$ ), and to the number of branching points in the main stem ( $r = 0.383$ ,  $P = 0.017$ ,  $n = 38$ ) and, being a linear dimension of the plant, will be proportional to plant biomass (White & Harper 1970). All reproductive components were measured at Cerro Garnica, and flower size, number of developed seeds and number of aborted seeds was recorded at San Jose. In addition, we assessed whether female flowers produce pollen by examining the anthers of female flower buds (three buds/plant) under a microscope on six plants at both populations.

Floral display, early and late fruit production (*i.e.* developing and ripening fruits) were compared between female and hermaphrodite individuals using a general linear model with morph as a fixed factor. For floral display, census day (dates 1–6, random factor) and the interaction morph  $\times$  census day were included in the model. Differences in corolla diameter, corolla length and in the number of viable and aborted seeds among the two morphs were assessed using a linear mixed model, with morph and population as fixed effects and plant nested within morph as a random effect. The interaction morph  $\times$  population was included in the models. Results of corolla diameter and length are qualitatively the same, and we only report statistical results of the former. Differences in fruit mass were analysed with a linear mixed model, using morph as fixed effect and plant nested within morph as random effect. When necessary, the natural logarithm of data was used to meet the assumptions of the analysis. Comparisons were made between female and fruiting hermaphrodites, except for floral display and corolla diameter, which also included non-fruiting hermaphrodites. The relation between height (predicting variable) and the number of flowers and fruits produced (early and late) per plant was assessed with linear regression. Hermaphrodites that did not mature fruits were not considered in the relationship between height and late fruit production because they can possibly be canalised males and would mask any relationship. To meet the assumptions of the test, data were log-transformed.

We assessed pollen limitation of females and hermaphrodites at both populations by comparing fruit set and seed number between pollen supplementation and open pollination treat-

ments. We selected 13 plants per morph per population, and on each plant we tagged between four and six recently opened flowers that were left open to pollinators. On the same plants, we manually pollen-supplemented the same number of flowers per plant with a mixture of pollen from two different donors located at least 10 m away from the recipient plant. Fruit set (proportion of treated flowers that produced fruits) between open-pollinated and pollen-supplemented treatments was compared with a general linear model, with morph as fixed factor and plant as random factor. To assess the capacity to produce seeds autonomously in hermaphrodite plants, 12 plants per population were selected and between two and eight buds per plant, depending on flower availability, were tagged and covered with mesh bags. During development, fruits are green, gradually changing to brown and finally turning black and soft when they are ripe. We collected the fruits produced when they were turning black after *ca.* 10 weeks, and estimated fruit set. Most of the plants used for the pollination treatments at San Jose were lost due to human disturbance, so we could only obtain results for the Garnica population. Finally, given that apomixis may reduce the potential effects of pollen limitation on female plants, in Garnica we included an apomixis treatment to explore if females can produce asexual seeds. For this, female buds were covered with mesh bags until flowers wilted to record fruit production (12 plants, six flowers per plant). Because genetic analyses of the progeny are needed to confirm apomixis, we refer to this treatment as putative apomixis.

### Offspring performance

In Garnica, seeds were collected from female and fruiting hermaphrodite plants to evaluate in a shade house (60% shade cloth) at the main campus of the Universidad Michoacana de San Nicolás de Hidalgo (Morelia, Michoacán), and assess whether there are differences in germination and early seedling growth between morphs. Mature fruits (black) were left at room temperature for 15 days until fruit pulp was dry, after which they were planted in individual 10-cm diameter pots. A total of 125 seeds from ten female plants and 73 seeds from nine hermaphrodites were planted in commercial soil. Pots were examined every other day and the soil was kept moist at all times. In addition, seeds from females produced by putative apomixis ( $n = 79$ ) were planted to evaluate their viability. Seed germination was evaluated as the emergence of cotyledons. Seed germination began 3 weeks after planting, and was monitored for the next 40 days. Seedling performance was examined after seed germination began in 14 seedlings of each morph, from six female and five hermaphrodite plants, by recording seedling height from 23 March, every week for 19 weeks. Proportion of seed germination was compared between female and hermaphrodite maternal plants using a general linear model, with morph as fixed factor. Seedling height at 19 weeks was compared with a linear mixed model, using morph as fixed effect and plant nested within morph as random effect.

### Nectar production and concentration

During October 2009, accumulated nectar production and concentration were estimated at Cerro Garnica in three plants of each morph (three flowers/plant). Each flower was bagged

before opening, and measured from the first day it opened, once per day for three consecutive days, replacing the bag after each measurement. Flowers open in the morning and last for 4–5 days (E. Cuevas, personal observation). Measurements were carried out between 09:00 and 11:00 h. Nectar volume was measured with calibrated micropipettes (5  $\mu$ l) and percentage sugar concentration was measured with a hand refractometer (Atago, Tokyo, Japan). A linear mixed model was used to test for differences between morphs and among days for nectar production and concentration, with morph and day used as fixed effects, plant nested within morph, and flower nested within plant nested within morph as random effects. The interaction day  $\times$  morph was included.

### Flower visitors

We recorded flower visits during 2008 and 2009 at the Garnica population on three sampling occasions each year (2008: 23 October, 5 and 27 November; 2009: 21 August, 20 September and 16 November). On each date, between 08:00 and 14:00 h, observation periods of 15 min were carried out on two to six randomly chosen plants of both morphs on sunny days, recording the number of pollinator visits and the total number of open flowers of the focal plants. For each pollinator that visited a focal plant, we recorded the pollinator type (morphospecies) and the number of flowers visited. A few individuals of each pollinator species were collected for later identification. In 2008 we carried out observations for a total of 8 h (32 periods of 15 min) and in 2009 for a total of 4.25 h (17 periods). The total number of visits and the number of pollinators attracted to hermaphrodite and female plants were compared using a linear mixed model, with morph, sampling dates and year as factors, and the number of open flowers as a covariate. Because dates varied among years, the sampling start and end were earlier in 2009, and sampling date was nested within year as a random effect. To examine whether visitation patterns varied among years, the interaction morph  $\times$  year was included in the model. The natural logarithms of number of visits, pollinators and flowers were used to meet the assumptions of the test. Results for the number of visits and pollinators do not differ qualitatively, and thus we only report the results for the number of visits to plants.

All statistical analyses were performed using SPSS 12.0.1 (SPSS Inc., Chicago, IL, USA). Means  $\pm$  SE are reported throughout. The population is specified when variables were studied at both populations, otherwise results correspond to the Garnica population.

## RESULTS

### Sex ratio and reproductive parameters

The morphological sex ratio (*i.e.* hermaphrodite and female) in San Jose was not significantly different from the expected 1:1 ( $\chi^2 = 1.47$ ,  $P = 0.22$ , female to hermaphrodite sex ratio = 1:1.22,  $n = 153$ ). In Garnica the sex ratio was biased towards hermaphrodites ( $\chi^2 = 4.98$ ,  $P = 0.02$ , female to hermaphrodite sex ratio = 1:1.55,  $n = 107$ ). The proportions of hermaphrodites that produced fruits were 0.47 at San Jose ( $n = 46$ ) and 0.38 at Garnica ( $n = 63$ ), giving final female:non-fruiting hermaphrodite:fruiting hermaphrodite ratios of 1:0.65:0.57 and

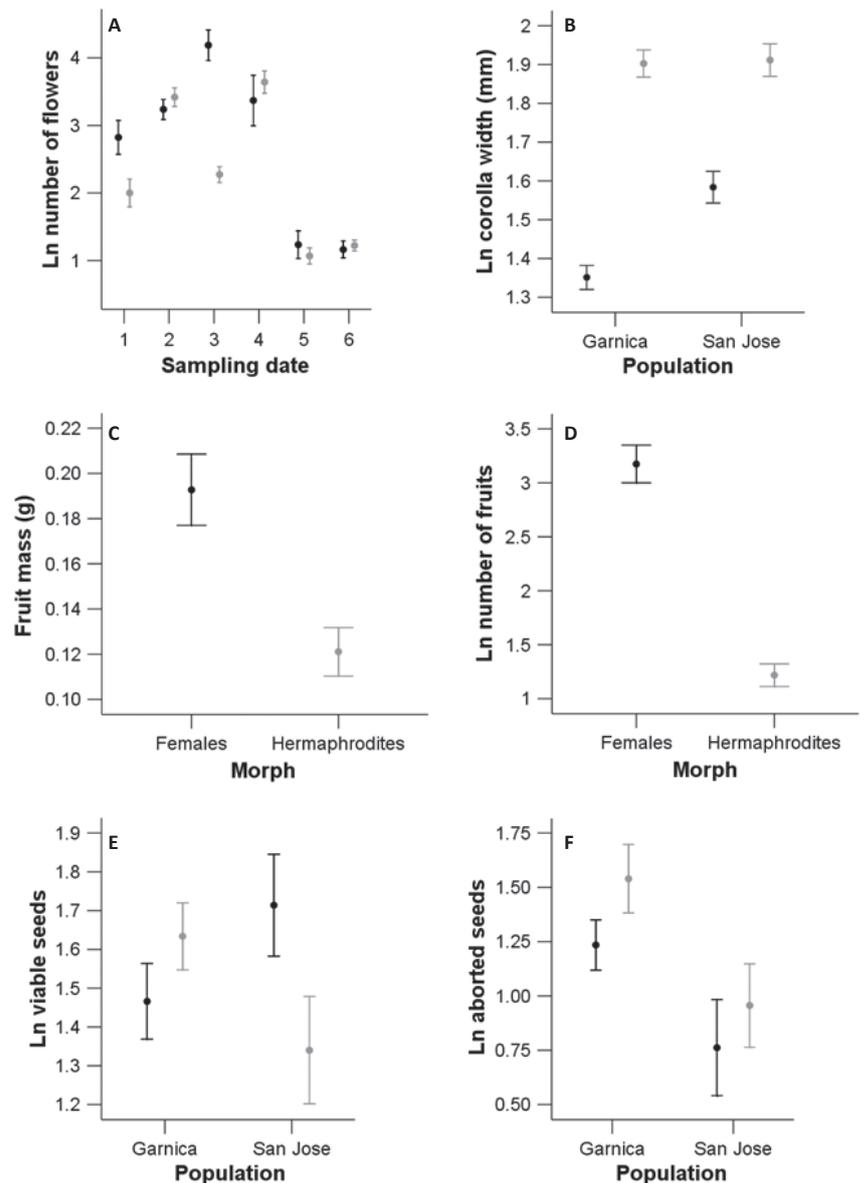
1:0.96:0.59, respectively. Floral display differed between dates ( $F_{5,5} = 8.063$ ,  $P = 0.019$ ) and was significantly larger in female plants than in hermaphrodite plants at some stages of the flowering season (morph:  $F_{1,5.2} = 1.404$ ,  $P = 0.287$ ; morph  $\times$  date interaction:  $F_{5,204} = 11.869$ ,  $P < 0.001$ ; Fig. 1A). In contrast, corolla diameter was significantly larger in hermaphrodite plants ( $F_{1,85} = 59.628$ ,  $P < 0.001$ ), and corollas of female plants tended to be larger in San Jose than in Garnica (population:  $F_{1,85} = 4.041$ ,  $P = 0.048$ , morph  $\times$  population  $F_{1,85} = 3.854$ ,  $P = 0.053$ ; Fig. 1B). Fruit mass, early and late fruit production were significantly higher in female plants ( $F_{1,14.1} = 10.137$ ,  $P = 0.007$ ;  $F_{1,51} = 20.661$ ,  $P < 0.001$ ;  $F_{1,63} = 32.262$ ,  $P < 0.001$ , respectively; Fig. 1C and D), but the number of viable and aborted seeds per fruit was not significantly different between morphs ( $F_{1,23.7} = 0.503$ ,  $P = 0.485$  and  $F_{1,21} = 0.409$ ,  $P = 0.529$ , respectively) nor between populations ( $F_{1,23.7} = 0.184$ ,  $P = 0.672$  and  $F_{1,21} = 3.825$ ,  $P = 0.064$ , respectively; Fig. 1E and F).

In hermaphrodite individuals, plant height was positively related to the number of flowers ( $F_{1,17} = 8.15$ ,  $P = 0.011$ ,  $r^2 = 0.32$ ; Fig. 2A), to early ( $F_{1,17} = 24.3$ ,  $P < 0.001$ ,  $r^2 = 0.6$ ; Fig. 2C) and final ( $F_{1,6} = 7.06$ ,  $P = 0.038$ ,  $r^2 = 0.54$ ; Fig. 2E) fruit production. In female individuals, however, plant height was related to the number of flowers ( $F_{1,22} = 5$ ,  $P = 0.03$ ,  $r^2 = 0.18$ , coefficient estimate  $\pm$  SE =  $1.02 \pm 0.46$ ; Fig. 2B) but not to fruit production ( $F_{1,18} = 0.85$ ,  $P = 0.37$ ; Fig. 2D). In hermaphrodites, with increasing size, fruit production increased more (coefficients: early =  $1.73 \pm 0.35$ , late =  $1.91 \pm 0.72$ ) than flower production (coefficient =  $0.68 \pm 0.24$ ). Assuming that pollen production per flower remains constant, this would suggest that with increasing resource status, the allocation to the female function increases more than the allocation to the male function in hermaphrodites, whereas in females, only the allocation to the number of flowers increases with resource status.

We found no pollen grains in the anthers of female flowers. In females, fruit set did not differ between open-pollinated ( $8.3 \pm 3.6\%$ ) and pollen-supplemented ( $13.5 \pm 6.08\%$ ) flowers ( $F_{1,12} = 0.431$ ,  $P = 0.524$ ). However, pollen-supplemented flowers produced significantly more developed seeds per fruit ( $6.1 \pm 0.59$ ) than control open-pollinated flowers ( $2.8 \pm 0.73$ ,  $F_{1,12} = 11.849$ ,  $P = 0.005$ ). In hermaphrodites, no fruit production was obtained from control and pollen-supplemented flowers. Two out of 12 hermaphroditic plants whose flowers were excluded from pollinators produced fruits. These two plants had a fruit set of 20%, producing on average five developed seeds per fruit. Four of the 12 female plants whose flowers were excluded from pollinators produced fruits by putative apomixis and had a fruit set of 21%. Furthermore, the number of developed seeds produced by putative apomixis ( $4.0 \pm 1.67$ ) did not differ from the number produced by female fruits under natural pollination ( $4.03 \pm 0.45$  in Garnica,  $5.31 \pm 0.84$  in San Jose).

### Offspring performance

Percentage germination of seeds derived from female plants ( $41.2 \pm 4.9$ ) was not significantly different from that of fruiting hermaphrodite plants ( $41.8 \pm 10.3$ ,  $F_{1,16} = 0.002$ ,  $P = 0.967$ ). Seedlings from female individuals were also similar in height to those from hermaphrodite individuals after 19 weeks ( $23.74 \pm 1.57$  mm,  $F_{1,7.7} = 0.045$ ,  $P = 0.837$ ). Finally, for females, 27% ( $\pm 2.7$ ) of the seeds produced by putative apomixis germinated.



**Fig. 1.** Reproductive components of hermaphrodite and female plants of the subdioecious *Fuchsia microphylla*. A: Floral display: number of flowers per plant per sampling date (between September 2008 and January 2009). B: Corolla width at two populations. C: Fruit mass. D: Late fruit production: number of fruits per plant. E: Number of developed seeds per fruit in two populations. F: Number of aborted seeds per fruit in two populations. Black bars – female plants, grey bars – hermaphrodite plants. In (A) and (B), hermaphrodites include fruiting and non-fruiting plants. Data shown are means  $\pm$  SE.

### Nectar production and concentration

Hermaphrodite and female flowers produced similar amounts of nectar ( $4.37 \pm 0.97 \mu\text{l}$  and  $3.58 \pm 0.56 \mu\text{l}$ , respectively,  $F_{1,10.9} = 0.547$ ,  $P = 0.475$ ; Fig. 3A). Nectar concentration did not differ significantly between hermaphrodite and female flowers, although it tended to be higher in hermaphrodites ( $F_{1,8.3} = 3.945$ ,  $P = 0.081$ ; Fig. 3B). Nectar volume increased significantly as flowers aged ( $F_{2,32.6} = 99.724$ ,  $P < 0.01$ ), while nectar concentration remained constant ( $F_{2,40.6} = 0.547$ ,  $P = 0.583$ ). This was consistent for both morphs (interaction day  $\times$  morph, volume:  $F_{2,32.6} = 0.246$ ,  $P = 0.784$ ; concentration:  $F_{2,40.6} = 0.005$ ,  $P = 0.995$ ; Fig. 3A and B).

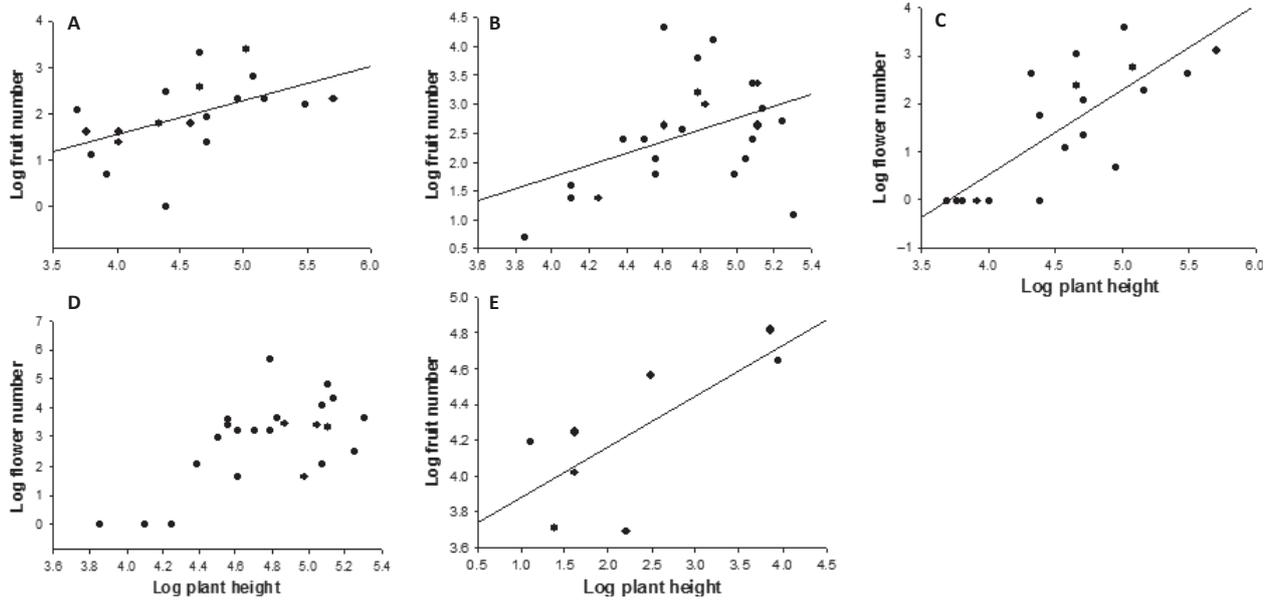
### Flower visitors

Except for two visits from wasps, the bees *Deltoptila* sp. and *Bombus ephippiatus* were the only floral visitors recorded. Both species made contact with stamens and stigmas of flowers while

visiting. The number of visits to flowers increased with the number of open flowers on individuals ( $F_{1,41.5} = 18.511$ ,  $P < 0.001$ ). The number of visits did not differ between hermaphrodite and female plants or between years (plants:  $F_{1,40.5} = 0.060$ ,  $P = 0.808$ ; years:  $F_{1,4.3} = 0.166$ ,  $P = 0.703$ ). Nevertheless, visitation patterns to morphs differed between years (interaction morph  $\times$  year  $F_{1,40.4} = 6.432$ ,  $P = 0.015$ ; Fig. 3C). Analysis of variance with the same explaining variables but for years and for morphs separately show that in 2008 hermaphrodites received more visits than females and that females received more visits in 2009 than in 2008. In 2008, visitation patterns were driven by *Deltoptila* sp., which accounted for 85% of the visitors to plants, and 77% of the visits to flowers. In 2009 *B. ephippiatus*, accounted for 66% of the visitors, and 62% of the visits.

### DISCUSSION

We studied the reproduction of two populations of the subdioecious shrub *F. microphylla*. The number of developed seeds



**Fig. 2.** Relationship between height and flower (A, B) and fruit (C, D, E) production of hermaphrodite (left) and female (right) plants of the subdioecious *Fuchsia microphylla*. (C) and (D) show early fruit production, (E) shows late fruit production.

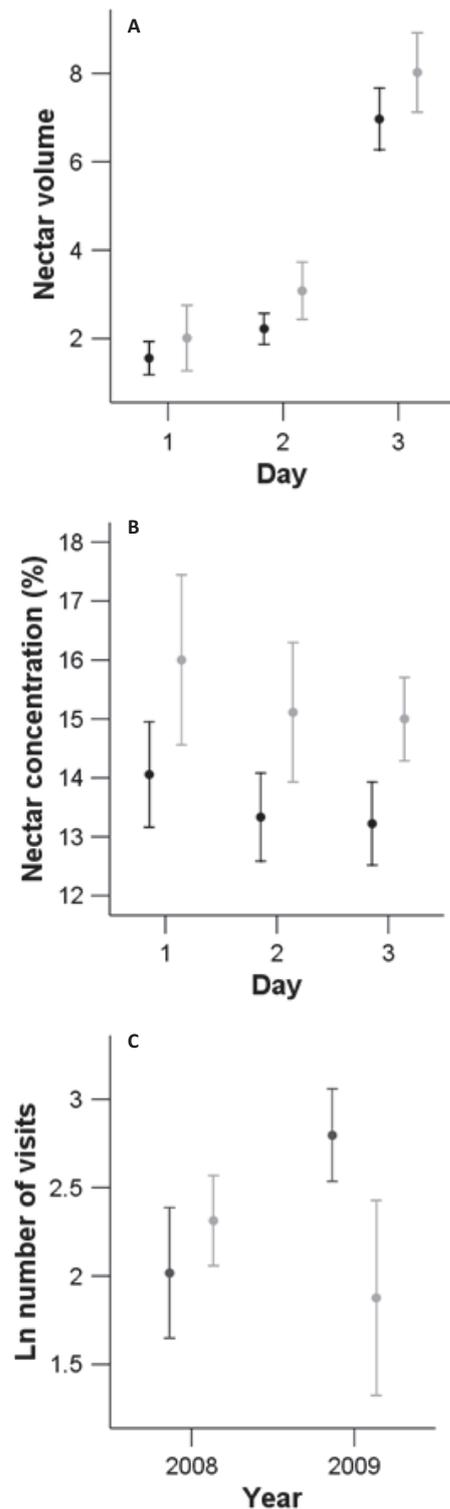
per fruit, and the percentage of germinated seeds did not differ between females and hermaphrodites; however, females produced on average 16.8 times more fruits than fruiting hermaphrodites ( $27.31 \pm 8.58$  versus  $1.63 \pm 0.39$ ). Therefore relative female fitness of hermaphrodites and females can be estimated from the mean number of fruits produced per plant. Thus, as expected for dimorphic species with female individuals, we found a large fertility advantage in female plants (Shykoff *et al.* 2003). Nectar volume and concentration were similar between morphs, but floral display and flower size differed. Pollinators did not show consistent morph preferences. Interestingly, females and hermaphrodites can set low and similar levels of fruit in the absence of pollinators. We discuss our findings in relation to the breeding system of this species.

We estimated the fertility advantage of female plants from the absolute number of fruits produced per plant. This difference also appears to be large in relative terms, hermaphrodites having to produce more flowers for each produced fruit: assuming total flower production could be estimated from the sum of the average floral display of each sampling occasion, fruit set is ten times higher in female plants (15.7% versus 1.5%). The female fertility advantage expected in sexually dimorphic species has been suggested to arise through the reallocation of resources previously used in pollen production or through inbreeding avoidance (Charlesworth & Charlesworth 1978; Shykoff *et al.* 2003). Female compensation was detected in the number of flowers, which differs from that reported for other subdioecious (Ramsey & Vaughton 2001; Aguirre *et al.* 2007) and dioecious species (Vaughton & Ramsey 1998 and references therein) in which hermaphrodites or males have larger floral displays, but is similar to reports of gynodioecious species (Shykoff *et al.* 2003; Ashman 2006). A higher number of flowers allows setting of a higher number of fruits. Here, hermaphrodites produce perfect flowers, which require more resources than unisexual flowers. This may explain the occurrence of female compensation at this level. In species in which hermaphrodites produce both male and female or perfect flow-

ers, this morph can invest more in the male function by having larger floral displays of male flowers.

Preliminary ovule counts suggest that female compensation also occurs in the number of ovules (E. Cuevas, unpublished results). The lack of female compensation in the number of seeds per fruit in the studied populations may be due to pollen limitation at this level, as females doubled the number of seeds produced per fruit following pollen supplementation, whereas hermaphrodite flowers did not produce fruits. The low fruit production of hermaphrodites under all pollination treatments suggests that fruit production in hermaphrodites is not limited by pollen. Fruit abortion appears to be higher for hermaphrodite than for female plants, considering the difference between early and late fruit production (fruit abortion of 91.2% and 60.1%, respectively). Therefore, the presence of inbreeding depression in offspring of fruiting hermaphrodites (Arroyo & Raven 1975; Raven 1979; Ramsey *et al.* 2006) cannot be discounted as partly accounting for the female advantage observed.

Despite the low fruit production, hermaphrodites were capable of producing fruits in the absence of pollinators. It is uncertain if this is through autonomous selfing or apomixis. These two reproduction mechanisms would result in different genetic scenarios and may affect reproductive assurance. If seed set is caused by selfing, some level of inbreeding depression is expected. In contrast, if caused by apomixis, then the seeds would be as heterozygous as the maternal plant. A relevant point is that females are putatively capable of setting seed *via* apomixis, which can reduce the effects of pollen limitation. To our knowledge this would be the first report of apomixis in the Onagraceae (see Raven 1979). Although the unequivocal confirmation of apomixis requires genetic and cytological evidence (Nogler 1984), its prevalence is underestimated, and a number of apomictic plant species are dioecious or self-incompatible, or belong to genera whose species predominantly exhibit self-incompatibility, dioecy or heterostyly (Bicknell & Koltunow 2004). Thus, it is a plausible mode of reproductive assurance that may be more common than currently known in species



**Fig. 3.** Nectar measurements and pollinator visitation of female and hermaphrodite plants of the subdioecious *Fuchsia microphylla*. A: Accumulated nectar production of flowers on 3 days. B: Nectar concentration of flowers on 3 days. C: Number of pollinator visits to flowers in 2 years on hermaphrodite and female plants; after considering the effect of the number of flowers, which on average is higher in female plants, only the 2008 difference between morphs is significant; see main text for statistical results. Black bars – female plants, grey bars – hermaphrodite plants. Data shown are means  $\pm$  SE.

with mechanisms that limit self-fertilisation, and may help to explain the persistence of dimorphic systems in periods in which cross-pollination fails.

#### Flower visitors

The same bee species dominated as pollinators in the 2 years of sampling, and no consistent differences in visitation were detected for the different morphs. However, we found variation in visitation to morphs among years, and in the relative contribution of visits to *F. microphylla* by the two bee species. Contrary to previous studies in other populations (Arizmendi *et al.* 1996), we did not register hummingbird visits, despite the fact that they were visiting other plant species at the sites. Hummingbird pollination might be more variable than that of the other species and may depend on the availability of other flowering resources in the area. Visitation could naturally vary during the flowering season and result in variation in pollination limitation.

#### The ecological context and subdioecy

Despite the occurrence of pollen limitation in the number of seeds per fruit in females and seed production in the absence of pollinators in hermaphrodites, given the magnitude of the female advantage it is unlikely that these conditions contribute to the stable existence of subdioecy in this species, as found in theoretical work (Ehlers & Bataillon 2007). Even under the unlikely scenario of complete pollinator limitation, some female advantage is expected, as fruit set in both morphs was very similar in the pollinator exclusion experiments, but females have larger floral displays. Consistent with previous work on subdioecious species, we recorded a slightly higher proportion of hermaphrodites than females at both sites (Arroyo & Raven 1975; Lloyd 1976; Delph & Wolf 2005). Assuming nuclear inheritance of male sterility (Arroyo & Raven 1975), if females were the heterogametic sex, then offspring of females would segregate 50:50 for each sex, whereas hermaphrodite plants would produce only hermaphrodites. This would result in sex ratios similar to those found here (in San Jose 55% and in Garnica 60% of individuals being hermaphrodites) and by Arroyo & Raven (1975) in plants grown from a mixed sample of seeds from a population. In terms of equilibrium sex ratios, assuming nuclear inheritance of male sterility and no differences in longevity and mortality (Lloyd 1976), the female advantage would need to be reduced from 16.8 to 2.1 at Garnica, considering that only 38% of hermaphrodites produced fruits. Given the low fruit production in hermaphrodites in this system it is unlikely that the sex ratios are at equilibrium. Based on the apparent hermaphrodite flower morphology of this morph, together with highly reduced fruit production, and given that no dioecious populations have been reported, we believe that subdioecy in *F. microphylla* is a transitional state towards dioecy. Furthermore, the low and variable fruit production among individuals in hermaphrodites suggests the presence of female sterility and therefore of canalised males (Arroyo & Raven 1975). This is consistent with the expected frequency-dependent selection for enhanced male function in hermaphrodites in the transition from gynodioecy to dioecy (Charlesworth 1989).

On the other hand, the higher increase in fruit production than flower production with increasing plant size in her-

maphrodites suggests a higher investment in the female function with increasing resource status in hermaphrodites, but not in females, as the gender plasticity hypothesis proposes (Delph & Wolf 2005). In other *Fuchsia* species, hermaphrodite fruit production has been found to be a plastic response in relation to the abiotic environment (Atsatt & Rundel 1982). Further research is needed to investigate if some hermaphrodites are in fact canalised males, and if so, the relative fitness of males and hermaphrodites. If all hermaphrodites can produce fruits depending on their resource status, and at no cost to the male function, hermaphrodites may persist in the population. It is plausible that hermaphrodites set fruit because it is not so disadvantageous and further female function reducing modifier alleles are not selected; thus, males occasionally make fruits (Charlesworth 1989). Contrasting forces may drive breeding system evolution to the subdioecious state at which populations of different species are currently (e.g. Spigler & Ashman 2011; Vaughton &

Ramsey 2012). Moreover, study of the ecological context in more populations of this species, together with information on the geographic range of the species and a phylogenetic approach reconstructing ancestral states of breeding systems in this group, will be essential to shed light on the evolution of the breeding system of this species.

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## REFERENCES

- Aguirre A., Vallejo-Marin M., Salazar-Goroztieta L., Arias D.M., Dirzo R. (2007) Variation in sexual expression in *Jacaratia mexicana* (Caricaceae) in southern Mexico: frequency and relative seed performance of fruit-producing males. *Biotropica*, **39**, 79–86.
- Arizmendi M.C., Dominguez C.A., Dirzo R. (1996) The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plant species. *Functional Ecology*, **10**, 119–127.
- Arroyo M.T.K., Raven P.H. (1975) Evolution of subdioecy in morphologically gynodioecious species of *Fuchsia* sect *Encliandra* (Onagraceae). *Evolution*, **29**, 500–511.
- Ashman T.L. (2006) The evolution of separate sexes: a focus on the ecological context. In: Harder L.D., Barrett S.C.H. (Eds), *Ecology and Evolution of Flowers*. Oxford University Press; Oxford, UK, pp 204–222.
- Ashman T.L., Stanton M. (1991) Seasonal-variation in pollination dynamics of sexually dimorphic *Sidalcea oregana* ssp *spicata* (Malvaceae). *Ecology*, **72**, 993–1003.
- Ashman T.L., Swetz J., Shivittz S. (2000) Understanding the basis of pollinator selectivity in sexually dimorphic *Fragaria virginiana*. *Oikos*, **90**, 347–356.
- Atsatt P.R., Rundel P.W. (1982) Pollinator maintenance vs fruit production – partitioned reproductive effort in subdioecious *Fuchsia lycioides*. *Annals of the Missouri Botanical Garden*, **69**, 199–208.
- Barrett S.C.H., Case A.L., Peters G.B. (1999) Gender modification and resource allocation in subdioecious *Wurmbea dioica* (Colchicaceae). *Journal of Ecology*, **87**, 123–137.
- Berry P.E., Hahn W.J., Sytsma K.J., Hall J.C., Mast A. (2004) Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. *American Journal of Botany*, **91**, 601–614.
- Bicknell R.A., Koltunow A.M. (2004) Understanding apomixis: recent advances and remaining conundrums. *The Plant Cell*, **16**, S228–S245.
- Breedlove D.E. (1969) The systematics of *Fuchsia* section *Encliandra* (Onagraceae). University of California Publications in Botany, **53**, 1–69.
- Charlesworth D. (1989) Allocation to male and female function in hermaphrodites, in sexually polymorphic populations. *Journal of Theoretical Biology*, **139**, 327–342.
- Charlesworth B., Charlesworth D. (1978) A model for the evolution of dioecy and gynodioecy. *The American Naturalist*, **112**, 975–997.
- Cuevas E., Lopez S. (2011) Sex ratio and sex-specific latitudinal variation in floral characteristics of gynodioecious *Kallstroemia grandiflora* (Zygophyllaceae) in Mexico. *Biotropica*, **43**, 317–323.
- Delph L.F. (1990) Sex-differential resource-allocation patterns in the subdioecious shrub *Hebe subalpina*. *Ecology*, **71**, 1342–1351.
- Delph L.F. (2003) Sexual dimorphism in gender plasticity and its consequences for breeding system evolution. *Evolution and Development*, **5**, 34–39.
- Delph L.F., Lively C.M. (1992) Pollinator visitation, floral display, and nectar production of the sexual morphs of a gynodioecious shrub. *Oikos*, **63**, 161–170.
- Delph L.F., Wolf D.E. (2005) Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. *New Phytologist*, **166**, 119–128.
- Ehlers B.K., Bataillon T. (2007) ‘Inconstant males’ and the maintenance of labile sex expression in subdioecious plants. *New Phytologist*, **174**, 194–211.
- Harper J., White J. (1974) The demography of plants. *Annual Review of Ecology and Systematics*, **5**, 419–463.
- Klinkhamer P.G.L., de Jong T.J., Metz H. (1997) Sex and size in cosexual plants. *Trends in Ecology & Evolution*, **12**, 260–265.
- Lewis D. (1941) Male sterility in natural populations of hermaphrodite plants. *New Phytologist*, **40**, 56–63.
- Lloyd D.G. (1975) Maintenance of gynodioecy and androdioecy in angiosperms. *Genetica*, **45**, 325–339.
- Lloyd D.G. (1976) Transmission of genes via pollen and ovules in gynodioecious angiosperms. *Theoretical Population Biology*, **9**, 299–316.
- Maurice S., Fleming T.H. (1995) The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphisms. *Oikos*, **74**, 55–60.
- Nogler G.A. (1984) Gametophytic apomixis. In: Johri B.M. (Ed.), *Embryology of Angiosperms*. Springer, Berlin, Germany, pp 475–518.
- Ramsey M., Vaughton G. (2001) Sex expression and sexual dimorphism in subdioecious *Wurmbea dioica* (Colchicaceae). *International Journal of Plant Sciences*, **162**, 589–597.
- Ramsey M., Vaughton G., Peakall R. (2006) Does inbreeding avoidance maintain gender dimorphism in *Wurmbea dioica* (Colchicaceae)? *Journal of Evolutionary Biology*, **19**, 1497–1506.
- Raven P.H. (1979) A survey of reproductive-biology in Onagraceae. *New Zealand Journal of Botany*, **17**, 575–593.
- Richards A.J. (2003) Apomixis in flowering plants: an overview. *Philosophical Transactions of the Royal Society B*, **358**, 1085–1093.
- Romero A. (1991) Contribución al conocimiento de los macromicetes del Parque Nacional Cerro GARCIA, Queréndaro-Cd. Hidalgo, Michoacán, México. Undergraduate Thesis, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mexico.
- Rzedowski G.C., Rzedowski J. (2001) *Flora fanerogámica del Valle de México*. Instituto de Ecología, A.C., Pátzcuaro, Michoacán, Mexico, pp 1406.
- Samson D.A., Werk K.S. (1986) Size-dependent effects in the analysis of reproductive effort in plants. *The American Naturalist*, **127**, 667–680.
- Shykoff J.A., Kolokotronis S.O., Collin C.L., Lopez-Villavicencio M. (2003) Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. *Oecologia*, **135**, 1–9.
- Spigler R.B., Ashman T.L. (2011) Sex ratio and subdioecy in *Fragaria virginiana*: the roles of plasticity and gene flow examined. *New Phytologist*, **190**, 1058–1068.
- Vaughton G., Ramsey M. (1998) Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia*, **115**, 93–101.
- Vaughton G., Ramsey M. (2012) Gender plasticity and sexual system stability in *Wurmbea*. *Annals of Botany*, **109**, 521–530.
- Venable D.L. (1992) Size–number trade-offs and the variation of seed size with plant resource status. *The American Naturalist*, **140**, 287–304.
- White J., Harper J. (1970) Correlated changes in plant size and number in plant populations. *Journal of Ecology*, **58**, 467–485.